JOINT ANNUAL REPORT OF

THE EUROPEAN UNION COMMUNITY REFERENCE LABORATORIES FOR RESIDUES

EUROPEAN COMMISSION CONTRACT PERIOD:

JULY 2000 - JUNE 2001

Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin

The four European Union Community Reference Laboratories (CRLs) for Residues.

Rijkinstituut voor Volksgezondheid en Milieu (RIVM)
Bilthoven, The Netherlands

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INTRODUCTION

The four European Union Community Reference Laboratories (CRLs) for residues have been designated in 1991 by the Council of the European Union as laid down in Council Decision 91/664/EEC of 11 December 1991. Their re-designation by the Council and their updated powers and operating conditions are laid down in Council Directive 96/23/EC of 29 April 1996.

Previous Joint Annual Reports of the four CRLs for residues have been published since 1995 over the contract periods August 1995 – July 1996, August 1996 – July 1998, August 1998 – July 1999 and August 1999 – June 2000. The last two reports have been published as one volume known as Commission document SANCO/2651/2001 [*5*].

The present Joint Annual Report of the four CRLs for residues (Commission document SANCO/338/2002) describes the activities during the contract period 1 July 2000 – 30 June 2001. It summarises the scientific activities of the four CRLs with respect to the improvement and implementation of analytical methodology and the scientific basis of residue control as based on Council Directive 96/23/EC of 1996. More details are given in a series of reports, proceedings and scientific publications listed in the list of references for each CRL. It is emphasised here that scientific progress was made each time in close co-operation with a group of colleagues, representing National Reference Laboratories (NRLs), Routine and/or Field laboratories (RFLs) and academics.

This report has been compiled and edited by CRL-RIVM in consultation with the other three CRLs and the Commission. It is available on request either directly from the Commission in Brussels, Belgium, or from any of the four CRLs for residues. Also the previous Joint Annual Reports are available from these sources as electronic files in Portable Document Format (PDF).

The Joint Annual Reports of the 4CRLs are for the purpose of summarising the activities of these laboratories and are not intended as presentations for financial inspections by the Commission as laid down in the contracts with the Commission. The CRLs are only partly financed by the Commission as laid down for the contract period in Commission Decision 2000/704/EC. The complementary costs are covered by the governments of the Netherlands, France, Italy and Germany, respectively.

RESIDUES WITHIN THE MANDATE OF THE CRLS AS LAID DOWN BY EU COUNCIL DIRECTIVE 96/23/EC OF 29 APRIL 1996 AND AS LISTED IN THAT DIRECTIVE.

CRL-RIVM

Stilbenes, stilbene derivatives and their salts and esters

Antithyroid agents

Steroids

Resorcylic Acid Lactones (RALs), including Zeranol

Sedatives

Mycotoxins

CRL-AFSSA-LMV

Antibacterial substances, including sulphonamides and quinolones

Dyes

Carbadox and Olaquindox

Chloramphenicol

CRL-BgVV

Beta-agonists

Anthelmintics

Anticoccidials, including nitroimidazoles

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

CRL-ISS

Carbamates and pyrethroids

Organochlorine compounds, including PCBs, polychloro-dibenzo-*p*-dioxins and polychlorodibenzofurans

Organophosphorus compounds

Chemical elements

CONTENT

Reports of the contract period

July 2000 to June 2001

Activities of the Community Reference Laboratory for residues at the Rijksinstituut voor Volksgezondheid en Milieu

CRL-RIVM, Bilthoven

covering the 7th financial contract period of 12 months

from July 2000 – June 2001.

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1 GENERAL

Identification of new and unknown compounds illegally used for growth promoting purposes (Group A substances).

Two sets of samples from "illegal cocktails" were received and analysed. All compounds detected were known for their use as anabolic agent.

EC - CRL –NRLs establishment of reference methods and minimum quality criteria for residues (co-ordination, co-operation and harmonisation).

Further support was given to the ongoing revision of Commission Decision 93/256/EEC and related Decisions with respect to performance criteria for analytical methods. Procedures for establishing Reference Methods and a list of Minimum Performance Limits were developed

and discussed with the NRLs. The practical implications were studied and (co)-published in the open scientific literature [*10, 13, 16*].

*EC/CRL – related **EC Bodies** (e.g. CVMP, EMEA, FVO, JRCs) and performance quality criteria (communication, co-ordination, co-operation and harmonisation).*

Extensive discussions took place with EMEA on the performance criteria for analytical methods to be submitted by the pharmaceutical industry for new veterinary drugs. The parallel EMEA-document (Draft Volume 8: "Establishment of maximum residue limits for residues of veterinary products in foodstuffs of animal origin: Development and validation of a proposed regulatory method") was discussed and commented. In the contract period full harmonisation of the two documents appeared not to be feasible.

*EC/CRL – related **International Bodies** (e.g. AOAC International, Eurachem, IUPAC, Codex, JECFA, IOC) and performance quality criteria (communication, co-ordination, co-operation and harmonisation).*

The efforts to actively introduce the EU concept of performance based criteria for analytical methods was continued in several discussions related to analytical methodology for residue analysis [*1 – 4, 19*]. International bodies like AOAC and Codex are taking a positive attitude towards this concept, however, full harmonisation between the EU and e.g. the USA and Canada remains a slow, but ongoing, process. The unfortunate circumstance that the draft document has still not yet been published in the contract period as an official Commission Decision has seriously hampered this process and challenged the creditability of the draft document in legal procedures against violators of the ban of EU non-authorised substances

[*14*].

Preparation of technical and financial reports on the activities of the CRL.

The technical and financial reports of the two contract periods (1999.08.01 – 2000.06.30 and 2000.07.01 - 2001.06.30) were prepared and have been submitted to the Commission.

2 METHODS DEVELOPMENT

Method development and improvement continued in several areas [*7, 8*]. A multi-residue method for the detection of residues of anabolic compounds in samples of faeces was developed and validated [*18*]. The method allows both the initial screening and subsequent confirmation of a wide range of compounds and was validated on the basis of the latest *draft* guidelines from the Commission (Document SANCO/1085/2000, previously known as SANCO/1805/2000).

The use of Supercritical Fluid Extraction (SFE) for the analyses of kidney fat and muscle tissue was further studied. Good results were obtained for gestagens in kidney fat and a range of androgenic and estrogenic compounds in muscle tissue, inclusive skin [*13*].

The existing multi-residue method for the analyses of tissues for anabolic steroids was validated for the screening and confirmation of a series of new matrices (muscle tissue from pork, turkey, fish, chicken etc.).

A protocol for the screening, quantification and confirmation of 8 sedatives in porcine kidney samples with LC-MSD was validated. The method was presented during the CRL-NRL workshop (October 2000) [*15*]. During this workshop analytical test samples (lyophilised spiked porcine liver) were made available to the participants.

Development of an analytical method for the detection and identification of somatotropines in biological samples was started. Activities focussed on LC-multiple MS (MS^n) analysis of PST preparations and a number of sample preparation techniques was tested.

To improve the flexibility of future analytical strategies, the use of individual analytical modules, to be combined into “tailor-made” analytical procedures, was further studied. This approach was further developed for the analyses of kidney fat for the presence of gestagens and will be the topic for the 2001 annual CRL-NRL workshop.

3 SUPPORTIVE RESEARCH WITHIN THE MANDATE OF THE CRL

Participation in a work programme for Zeranol metabolism (FAIR programme).

This study is still ongoing. NRLs were asked to submit relevant samples which were analysed for the presence of Zeranol and metabolites. Analyses to validate the immunoassays developed within this project were performed [*7*].

Animal experiments into the metabolism of different anabolic compounds and in support of the development of test and reference materials.

During this contract period one animal experiment was performed. Metabolism and biotransformation studies on the steroids methyltestosterone, methylboldenone and norethandrolone were performed.

*Participation in the European Consortium for Continuing Education for Advances in Meat Science and Technology **(ECCE-AMST)** project in co-operation with University of Utrecht, NL. (EC DG12 /5th Framework proposal in Thematic Networks).*

Some activities started during this period anticipating the approval of the proposal.

Participation in the European Consortium for Metrology in Qualitative Analysis

*(**MEQUALAN**) in co-operation with the University of Cordoba, ES. (EC DG12 /5th Framework Growth Programme).*

The project with 14 members from seven EU countries has started [*17*]. The objectives of the project are to develop a document supporting a practical metrological approach to the binary yes/no response in qualitative (bio)chemical analysis. Such responses are scarcely considered in classical metrology although they constitute a substantial part of the chemical information demands posed by industry and society, especially in the area of test results for banned substances. The kick-of meeting was held in Cordoba (ES), January 2001. A project related paper was presented at Paris, June 2001 [*16*].

*Participation in **ISOTRACE** project for the detection of illegal drugs by isotope ratio MS. (EC DG12 /5th Framework Measurement & Testing proposal)*

The project has started. Although the kick-of meeting already was held in May 2000, the necessary equipment has not yet been delivered. Subsequently, until the end of this contract period, progress is limited. A follow-up meeting was attended on 4-6 April 2001 in Barcelona (ES).

Measurement of hormonal anabolic agents (a.o. natural steroids and WTO dispute-related ones) in various food commodities from the EU market to establish the total daily dietary intake of such substances.

A study proposal was submitted to the Director-General of DG SANCO for additional financing of this work [*21*]. Financial support, however, was not made available [*22*]. Nevertheless, additional measurements with respect to the presence of natural hormones in meat are, at a limited scale, being performed. This activity is ongoing and definitively needs a higher Commission priority in perspective to future WTO hormone dispute cases. In such cases the situation on the EU market and e.g. the USA market have to be compared to actually assess consumer risks and hazards.

Investigation of residues of endogenous and/or exogenous anabolic compounds in meat products after preparation for consumption. Risk estimate-related task.

A series of samples of liver from retail shops was analysed for nortestosterone. The results will be published in the next contract period. The influence of processing remains to be studied. This activity is ongoing, partly in co-operation with international laboratories involved in testing for doping misuse in sports.

4 QUALITY ASSURANCE & QUALITY CONTROL

Maintenance of the in-house QA/QC activities, in consequence of the accreditation and GLP compliance of all analytical work done within the CRL, remained fully operational. The formal accreditation of the CRL continued and the GLP status remained “in compliance”. Due to cost-benefit considerations and to disputes with the Dutch GLP authority, it was decided,

however, to limit the GLP-status to studies related to formal arbitration cases. Maintenance of the GLP-status even for this purpose has to be reconsidered in the next contract period on the basis of the outcome of a full GLP re-inspection as scheduled for July 2001 by the Dutch GLP authority.

On behalf of the Board of Directors internal RIVM QA audits of the CRL were performed on 11 July 2000 and on 26 June 2001.

The EU-NRL inventory of infrastructure and QA systems for mycotoxin analysis is in progress.

5 TECHNICAL AND SCIENTIFIC SUPPORT TO NRLS AND THIRD COUNTRIES

Analyses of samples submitted by EU Member States in case of dispute between Member States or in case of analytical problems within a responsible NRL.

Assistance with analytical problems was given to two NRLs in EU Member states and three NRLs of third countries. A CRL-NRL workshop with the title "Residue analysis of sedatives, an analytical update" was organised at Bilthoven on 9-11 October 2000. An extensive documentation set was made available to the participants [*15*]. Participants were asked to analyse a set of lyophilised test materials with their "in house" method.

Several trainees visited the laboratory and received training on specific topics related to residue analysis. A more intense co-operation with several other research Institutes and Universities was discussed and announced (EuroResidue School). The special link with the Utrecht University, Faculty of Veterinary Medicine is strengthened by the part-time position of the CRL-Director as Professor on the chair of “Residues of Veterinary Drugs in Food of Animal Origin” [*9*].

The communication and co-operation with professionally related institutions was continued. Examples given are advisory tasks for the International Olympic Committee with regard to sports doping (Lausanne (CH) in December 2000 and Cologne (DE) in March 2001) for the Tulane University [*20*] and the US/FDA with regard to hormonal anabolic agents and

environmental endocrine disruptors (New Orleans/LA (USA) in October 2000 and Rockville/MD (USA) in October 2000).

A rather intensive and effective communication and co-operation has been started with lawyers and law enforcers organised across 4 EU Members States (OMLEC meetings at Maastricht (NL) in December 2000 and at Bilthoven in June 2001, the BULL seminar at Maastricht in April 2001 and a major appeal DES legal court case in Germany in September 2000).

The CRL participations to the above-mentioned events all took place on invitation and in all cases lectures were given about the EU CRL-NRL-RFL system and its evolution.

In view of the rapidly changing mycotoxin regulations in food and feed, a contract was in preparation with FAO to prepare a document with up-to-date information on worldwide regulations for mycotoxins. Part of this work is of relevance within the CRL tasks, as it relates to mycotoxins in food of animal origin or animal feed. This specific part will be carried out as a joint FAO/CRL exercise within the next contract period.

6 TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION

The contract studies for the Commission with regard to “hormones” residue testing of beef originating from the USA have been succesfully completed and reported in conjunction with the French NRL for residues at Nantes [*11*, *12*]. The Commission did not authorize the integral publication of the results in the scientific literature. However, on request of the Commission a few results have been published [*6, 10, 24*].

A formal Memorandum of Understanding (MoU) covering co-operation and mutual support has been established with the Commissions Joint Research Centre (JRC) “Institute of Health and Consumer Protection” (IHCP) at Ispra (IT) [*23*].

A similar MoU with the JRC “Institute for Reference Materials and Measurements” (IRMM) at Geel (BE) is in progress.

In the EMEA marketing authorization procedure for veterinary drug preparations containing hormonal steroids (e.g. progesterone, chlormadinone, flugestone and altrenogest) the CRL assisted DG SANCO on request with a series of written "second opinions" especially with regard to fitness-for-purpose of the "routine confirmatory method" as submitted by the veterinary drug manufacturer to the EMEA authorization file. Quite often this fitness was lacking.

The CRL participated in an EC-4CRL Management Board meeting at Rome (IT) on 29-30 May 2001.

On a continuous basis the CRL assisted the DG SANCO/FVO in their inspection missions, often *ad hoc around the clock* by telephone and e-mail advisory services to FVO inspectors on missions all over the world. See also Annex 2 "Audits".

On request of the Commission a start was made to evaluate the results of the National Residue Plans of the Member States. However, due to a too strict deadline this complex and major effort could not be completed in due time and will be continued in the next contract period.

7 PROFICIENCY TESTING AND REFERENCE MATERIALS

In July 2000 the results of the proficiency test "16beta-hydroxy-stanozolol in lyophilised bovine urine" were sent to the participants.

From the results it was concluded that about half of the Member States, at the time this proficiency test was organised, did not perform residue testing for this compound. It was recommended to undertake the necessary effort to further harmonise the National Residue Plans in this respect.

In September 2000 the samples for a proficiency test 17alpha-/17beta-boldenone and 17alpha-/17beta-nortestosterone were sent to the participants.

Activities with respect to reference materials during this period were focussed on incurred samples for the steroids Trenbolone, stanozolol, corticosteroids, nortestosterone and boldenone.

About 15 years ago RIVM/ARO prepared under contract with the Commission's BCR four batches of milk powder reference materials with certified contents of aflatoxin M_1. Now the stock of this very successful and worldwide used important reference material is coming to an end and the JRC-IRMM in Geel (BE) is in process to prepare a new stock.

In this process the CRL strongly supports the JRC and samples of milk powders supplied by JRC-IRMM were analysed to check their suitability. It was concluded that these candidate reference materials were suitable and preparations started for a contractual agreement with JRC-IRMM to carry out relevant homogeneity and stability experiments of these batches milk powder within the next contract period.

The supply of numerous reference samples and calibrants to NRLs and related laboratories continued as usual on a regular bases.

8 DOCUMENTATION SERVICES

The activities with respect to the collection, evaluation, digitalisation, filing and stock keeping of the analytical method standard operating protocols (SOPs) was continued, focussing on new approaches for dissemination of information through the internet. The RIVM/CRL website is under construction.

On request of the NRLs and related and / or equivalent laboratories the supply of scientific documents and operating protocols ("methods") continued on a regular bases. The majority of the documents were distributed by e-mail as Portable Document Format (PDF) copies. However, an appreciable number of requested documents had to be mailed as hard copy due to a lack of the receivers e-mail facilities.

9 ANNEXES

9.1 Annex 1 - Publications, Reports and Other Informative Material of the CRL related to the substances in its mandate

1. Arnold D, Stephany RW. Melengestrol acetate (MGA). In: Residues of some veterinary drugs in animals and foods. FAO Food and Nutrition paper 41/13. February 2000; 75–86. ISBN 92-5-104474-0.

2. Egmond HP van. Chapter 6. Mycotoxins: Detection, Reference Materials And Regulation. In: Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O. Introduction to food- and airborne fungi. Centraal Bureau voor Schimmelcultures, 6th edition, Utrecht NL, 2000; 332–338. ISBN 90-70351-42-0.

3. Egmond HP van, Dragacci S. Chapter 7. Liquid chromatographic method for aflatoxin M1 in milk. In: Trucksess MW, Pohland AE. Methods in Molecular Biology, volume 157: Mycotoxin Protocols; Humana Press, Totowa, NJ (USA). 2000; 59-69. ISBN 0-89603-623-5.

4. Egmond HP van, Jonker MA. Manuscript JECFA monograph on aflatoxin M_1. 56th Joint FAO/WHO Expert Committee on Food Additives. 12 January 2001; pp. 30

5. Gowik P [editor], Maris P, Stephany RW, Caroli S. Joint annual report on European Union Community Reference Laboratories for residues. European Commission contract period August 1998 - June 2000. Commission document SANCO / 2651 / 2001, July 2001; pp. 151 *(CRL-RIVM document 389002 114)*

6. Jégou B, Soto A, Sundlof S, Stephany R, Meyer H, Leffers H. General discussion: existing guidelines for the use of meat hormones and other food additives in Europe and USA. APMIS 109 suppl. 103 (2001) 551-556. ISBN 87-16-16462-8.

7. Kennedy DG, Sterk SS, Blokland MH, Van Ginkel LA van. Development, validation and harmonisation of screening and confirmatory tests to distinguish zeranol abuse from fusarium toxin contamination (EU FAIR5-PL97-3443 programme), Progress report of 2nd annual project group meeting on 11 May 2000 at Bilthoven (NL), pp. approx. 450.

8. Niesing W. The development of methods of analysis for corticosteroids in biological matrices *(in Dutch)* . Report HLO – Utrecht, Analytical Chemistry, November 2000; pp. 82

9. Stephany RW. "The residue in safe food: Tracking derailed traces" *(in Dutch).* Inaugural address on the occasion of acceptance of the Chair "Residues of veterinary drugs in food of animal origin" within the Department of the Science of Food of Animal Origin (VVDO), Faculty of Veterinary Medicine, Utrecht University, Utrecht (NL) 10 May 2001: pp. 13

10. Stephany RW. Hormones in meat: different approaches in the EU and in the USA. APMIS 109 suppl. 103 (2001) 357-364. ISBN 87-16-16462-8.

11. Stephany RW, André F. Results of "hormone" residue analyses of bovine meat and liver originating from the USA domestic market. CRL-RIVM document 389002 093; second interim report June 2000; pp. 38

12. Stephany RW, André F. Results of "hormone" residue analyses of bovine liver originating from the USA and imported into the EU as pet food. CRL-RIVM document 389002 092; third interim report June 2000; pp. 31

13. Stolker AAM, Stephany RW, Ginkel LA van. Identification of residues by LC-MS. The application of new EU guidelines. Analusis 28, 10 (2000) 947–961

14. Zoontjes PW. Schwillens PLWJ, Sterk SS, Ginkel LA van. Confirmation of dexamethasone in calf liver. CRL document 389002 101; 27 February 2001; pp.6. *(Confidential report.)*

15. CRL-NRL Workshop "Analysis of sedatives, an analytical update", 9 –11 October 2000 at RIVM, Bilthoven, NL. Binder with a collection of documents (SOPs, background information and literature) handed out to the participants during the workshop. *No report number* .

16. Stephany R.W. From measurements in the food area to interpretations: a risky business. Proceedings of the EU Conference "Challenge for Measurements. Environment, "Health, Safety. Paris (FR), 14-15 June 2001. CDROM publication of the French Institut National de l'Environnement Industriel et des Risques (INERIS), Verneuil-en-Halatte (FR). Homepage: http://www.ineris.fr.

17. Valcárcel M, Cárdenas S. Open call for suggestions by the MeQualAn consortium. Flyer pp. 4. University of Cordoba (ES), January 2001. E-mail: qa1meobj@uco.es

18. Herbold HA, Sterk SS, Stephany RW, Ginkel LA van. Screening of faeces for anabolic steroids using coupled-column HPLC and GC-MS. CRL-RIVM document 389002 100; February 2001, pp. 26.

19. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Evaluation of certain veterinary drug residues in food: 54th report of the JECFA: Production aid Melengestrol acetate (MGA). WHO technical report series 900, 2000, Geneva (CH), pg 64-82. ISBN 92-4-120900-3.

20. Stephany RW. Hormones in meat: a European Union horror story or a USA fairy tale? Abstract book Environmental Hormone Symposium 2000 Tulane University New Orleans (USA) 15 –18 October 2000, pp. 144

21. Letter 2000/0713 ARO of 27 June 2000 from R.W. Stephany (CRL) to R.J. Coleman (DGSANCO)

22. Letter D12785 of 26 October 2000 for R.J. Coleman (DGSANCO) to R.W. Stephany (CRL)

23. Mik G. de, McSweeney F. Collaboration Agreement No 17361-2000-12 S0SC ISP DE. Bilthoven (NL) and Ispra (IT), 2 April 2001.

24. Stephany RW. Hormones found in meat samples from regular controls within the European Union and from the US imports. Chemical Awareness, 9, July 5 th 2000, pg. 12-14. ISSN 1399-8315.

9.2 Annex 2 – Audits

Due to an inadequate budget within the CRL contract with the Commission for travel and subsistence, in this contract period the scheduled visits to NRLs had to be postponed.

The CRL assisted DG SANCO/FVO in its inspection mission to Australia from 9 - 21 November 2000 to evaluate the operation of controls over residues in products of animal origin and over the production of fresh meat, game meat, milk and milk products.

The full mission report (DG(SANCO)/ 1217/2000 – MR final) is available on the Internet as PDF document.

http://europa.eu.int/comm/food/fs/inspections/vi/reports/australia/vi_rep_aust_1217-2000_en.pdf

9.3 Annex 3 – Organigrams and Staff

Organigram and Staff of the CRL- RIVM

MANAGEMENT

Prof.Dr. R.W. Stephany	Director of the CRL- RIVM for residues,
	Head of the RIVM Laboratory for Residue Analysis (ARO).
	Head of the ARO Section for Informatics and Logistics.
Dr. L.A. van Ginkel	Deputy Director of the CRL- RIVM for residues.
	Deputy Head of RIVM/ARO.
	Head of the ARO Section of Veterinary Drugs and Medicines.
Ir. H.P. van Egmond	Head of the ARO Section of Natural Toxins and Nitro Substances.

ANALYTICAL SERVICES

Drs. S.S. Sterk	Scientist, Study-director (responsible for analytical services).
Drs. A.A.M. Stolker	Scientist.
H.A. Herbold	Senior Technician.
P.L.W.J. Schwillens	Senior Technician.
H.J. van Rossum	Senior Technician.
K.L. Wubs	Senior Technician.
M.H. Blokland	Technician.
P.W. Zoontjes	Technician.

DOCUMENTATION SERVICES

Drs. M.A. Jonker	Literature Scientist (a.o. responsible for documentation services).
C.G. van de Kamp	Senior Documentalist, Literature Scientist (a.o. responsible for maintenance and updating of Analytical Methods database).
J. ten Have	Documentation Supporter, Archiver .
A.A.M.M. Wilbers	Documentalist (responsible for maintenance and updating Reference Materials databases). Logistic Supporter (responsible for dispatch of Reference Materials).

QUALITY ASSURANCE

Dr. R.C. Schothorst	QA/QC & GLP Officer (responsible for QA/QC Infrastructure).
A. van der Berg	QA/QC Officer (a.o. coordinator maintenance of ISO 17025 status).

SUPPORT SERVICES

E. van Tamelen	Management Assistant.
M.A. Kartasasmita	Management Assistant, Secretary
E.Th. Sahertian	Information Technology and Automation Officer. (responsible for computer and LAN services).
M. van Tuyl	Amanuensis, Logistic Supporter.

9.4 Annex 4 – Workshops

Title:	Analysis of sedatives, an analytical update
	9 –11 October 2000 at RIVM, Bilthoven, NL
Participants:	15 from NRLs and 3 observers.
Workshop package:	see reference [*15*].

9.4.1 Conclusions and recommendations

- There is a need amongst the NRLs for information how to acquire standards for sedatives.
- There is a need for (incurred) reference materials and ring testing for in-house quality control.
- There is a need for information exchange between the NRLs, and between the CRL and NRLs, on positive findings for sedatives. Especially the compounds found, the amount and the species. This information can be used to agree upon the compounds to be screened for in the National Plans.
- For some NRLs a “hands on” training to get practical experience with the validated LC-MS(/MS) methods of the CRL is of interest.
- There is a need for documented information with respect to the stability of analytes in matrices and pure solutions.
- LC-(MS) seems to be the method of choice for the analysis of sedatives. All NRLs should have LC-MS at their disposition.
- The implementation of the SANCO/1805/2000 criteria document for Group B substances, like sedatives, seems to give no major problems.
- According to the NRLs one of the main tasks of the CRL should be the preparation of standards and quality control materials for in-house validation and quality assurance, inclusive the organisation of proficiency tests.
- The CRL should be a communication channel for information from- and to the NRLs. The organisation of workshops is also regarded as a good tool to come to good information exchange.

Final Report on the Activities of the Community Reference Laboratory for Residues of Beta-Agonists, Coccidiostats, Anthelmintics and NSAIDs for the Period of 1 July 2000 to 30 June 2001 CRL-BgVV, Berlin

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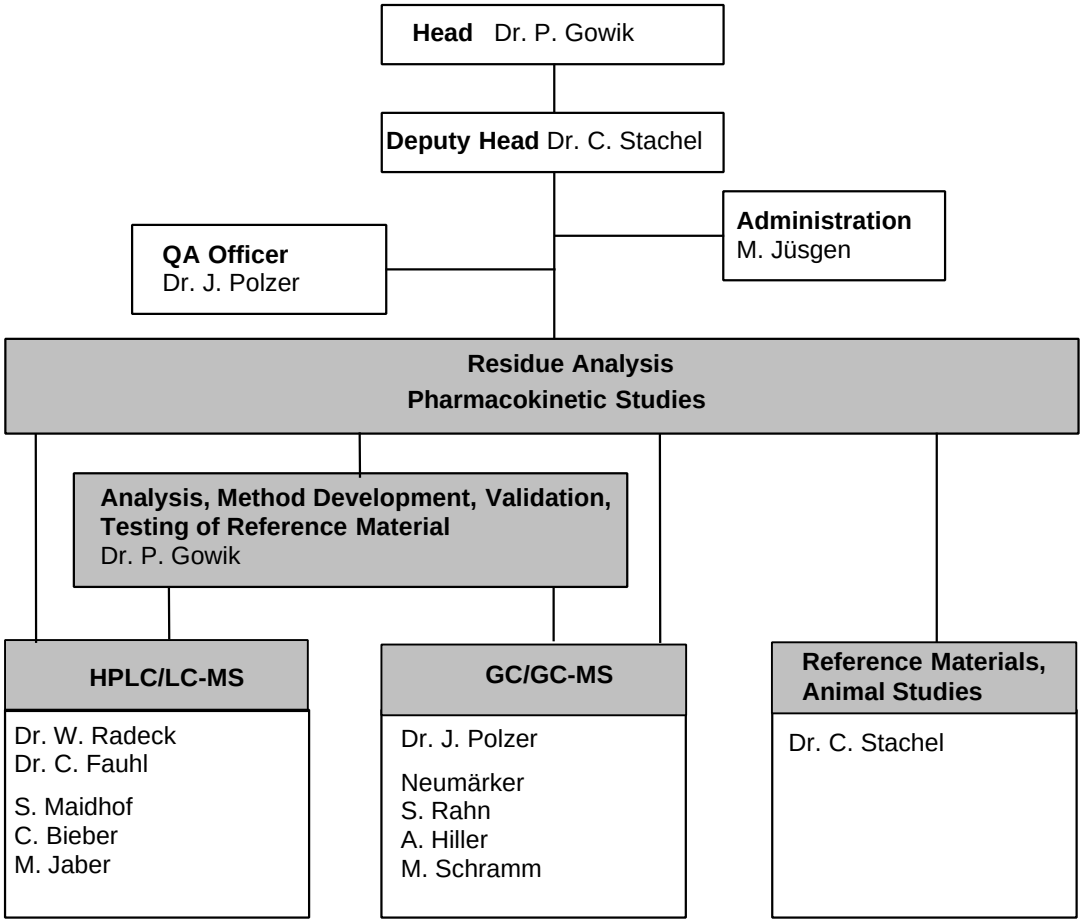
1 GENERAL

The CRL Berlin for residues of β-agonists, anticoccidials including nitroimidazoles, anthelmintics and non-steroidal anti-inflammatory drugs (NSAIDs) appertains to the division "Chemistry and Technology of Foods and Consumer Goods" of the BgVV. The analytical activities of the CRL Berlin are pursued by two specialised sub-units, one being responsible for GC and GC-MS, the other for HPLC and LC-MS methods, which are supplemented by a third sub-unit in charge of the preparation of incurred test materials to be used as in-house reference samples and for proficiency testing.

The staff of the CRL Berlin for residues consists of three scientists occupying the equivalent of 3 full-time posts, three technicians with 2.18 full-time equivalents and one person with one full-time equivalent for administration and translation. In total seven persons with 6.18 full-time equivalents are working for the CRL, supplemented by 5.67 members of BgVV staff including the head of the CRL/NRL, one full-time scientist and four technicians with 3.17 full-time equivalents. The divisions and competences within the CRL/NRL unit are shown in the organigram.



The activities listed in the following correspond to the duties and operating conditions of CRLs as laid down in Annex V, Chapter 2 of Council Directive 96/23/EC of 29 April 1996. The respective section of Council Directive 96/23/EC is indicated at the beginning of each chapter. Attention has to be drawn to the fact that the work of the CRL Berlin was considerably impaired during this reference period by the substantial shortage in the budget for consumables due to the increase of the staff's salaries while the total budget stayed fixed. Since the tasks, especially the provision of standard reference substances, increase continuously, this budget shortage for consumables had to be counterbalanced by the institute's budget, which however was not possible to a full extend.. As a consequence, the procuring of important deuterated internal standard substances had to be reduced to nearly zero.

2 GENERAL TASKS

- In cases of disputes or analytical problems concerning the identification and quantification of analytes, one official sample submitted by authorities from other EU Member States was analysed and the corresponding reports were submitted.

  Annex V, chapter 2, section 1 (h)

- The search and identification of unknown compounds illegally used for growth promoting purposes is considered an ongoing task. During this reference period the activities in this field were concentrated on Zilpaterol, a substance belonging to the beta-agonist group which seems to be a very potent drug for growth promoting purposes. Therefore and due to the fact that Zilpaterol is authorised in Mexico and South-Africa as a feed additive, it is a substance of high importance as regards the control of misuse in Europe. A dossier has been started with the available information, and regular internet enquiries were performed. In addition, the CRL Berlin is in contact with the NRL Bilthoven (RIVM) and the NRL Rome on this issue. A meeting took place at the BgVV with representatives of the NRL Bilthoven and the CRL Berlin, where further measures were agreed upon. After a comprehensive, long-lasting correspondence with the producer, a small amount of standard substance was put at the CRL's disposal for analytical purposes.

  Annex V, chapter 2, section 1 (a, j)

- The head of the CRL took part in the Commission meeting on the coordination system of the 4 CRLs, which this time took place in the Istituto Superiore di Sanità, Rome, from 29 to 30 May 2001. The CRL Director was furthermore involved in the finalising discussions on the “Draft of the Revision of Commission Decision SANCO/1805/2000”, created by a special working group of the Commission. Additionally, the Head of the CRL acts as a deputy representative for the German delegation in the Codex Committee on Methods of Analysis and Sampling.

  Annex V, chapter 2, section 1 (j)

- Involvement of the CRL in a project of the BgVV-department CRL/NRL aiming at the development of a software for the validation of test methods in accordance with SANCO/1805/2000. This software is intended to improve validation efforts and to harmonise the validation procedures in order to receive comparable proficiency data from the official residue control laboratories in Europe.

  Annex V, chapter 2, section 1 (a, c)

- Maintenance of the quality management and preparation of the forthcoming audit for the prolongation of the accreditation.

  Annex V, chapter 2, section 1 (a-l)

- The CRL management prepared a preliminary technical report on its activities during the contract period 2000-2001. Additionally, cost estimates and work plans for the next contract period and for a two-year contract period were prepared.

  Annex V, chapter 2, section 1 (k)

- From 20 November to 01 December 2000 the Head of the CRL participated as an external expert in a two-week inspection visit of the FVO to Argentina to check the Argentinian residue control in live animals and products thereof.

  Annex V, chapter 2, section 1 (j)

- From 07 May to 18 May 2001 the Head of the CRL participated as an official escort of the German government in a two-week inspection visit of the FVO to Germany to check the German residue control in live animals and products thereof.

3 DEVELOPMENT, OPTIMISATION AND VALIDATION OF ANALYTICAL METHODS

Annex V, chapter 2, section 1 (a, c, d)

3.1 Multi-residue method for the determination of acid NSAIDs

The validation of the HPLC-DAD method for the determination of acid non-steroidal anti-inflammatory drugs (NSAIDs) including flunixin hydroxide, phenylbutazone and its metabolite oxyphenbutazone and prodrug suxibuzone, carprofen, vedaprofen, tolfenamic acid, niflumic acid, mefenamic acid, flurbiprofen and diclofenac in milk of different species and with different fat contents was carried out. The method developed by the CRL Berlin proved to be rather robust against differences in fat content, storage temperature and duration, conditions of sample preparation like different quantities of extracting solvent, minor pH variations etc. However, the degree of robustness depends on the substance.

The MRL for tolfenamic acid in milk of 50 µg/kg can be controlled with this method, which has a working capacity of 25 to 5000 µg/kg. The CC_α determined for tolfenamic acid is 56.7 µg/l.

Unfortunately, it emerged during the validation study that the recovery and the within-laboratory reproducibility for flunixin hydroxide do not comply with the given criteria of Commission Decision 93/256/EC and of SANCO/1805/2000. Therefore an extra method to control the compliance with the MRL of flunixin hydroxide in milk has to be developed and validated.

No MRLS exist for the other substances so far, as they are either not authorised for milk-producing animals or not authorised for veterinary purposes at all. Therefore investigations were started in order to adjust the method to an LC-MSMS technique.

The validation was carried out with three different kinds of milk:

- cow milk with a low fat content (~ 3.5 %)
- cow milk with a high fat content (~ 5 %)
- buffalo milk with a high fat content (~ 9 %)

and a minimum of three concentration levels: 0.5, 1, 1.5 times MRL (TLF 50 µg/l and FLU-OH 40 µg/l) and 1, 1.5, 2 times MRPL for the other substances.

3.2 Multi-residue method for the determination of metamizole metabolites and ramifenazone (dipyrone)

The screening and confirmatory method on the basis of HPLC/DAD for the determination of 4-methylaminoantipyrine, ramifenazone and antipyrine as well as their metabolites in liver was validated on the basis of the revised criteria of SANCO/1805/2000. Dimethylaminoantipyrine was used as an internal standard.

A validated method for the determination of the metamizole metabolite 4-methylaminoantipyrine in liver does now exist. It could be shown that the method can also be used for the determination of 4-methylaminoantipyrine, ramifenazone and phenazone in kidney and plasma, as well as, in a slightly modified form, for muscle.

A validation for ramifenazone and phenazone according to the new criteria does not yet exist. So far, these two substances have been classified by EMEA as “no recommendation”. In residue control they therefore have to be treated like banned substances, i.e. *inter alia* that according to the new criteria a confirmation with LC/DAD is not sufficient, but a detection by means of mass spectrometric techniques is required. Therefore a separate validation has to be carried out for them.

Marker residue for metamizole: 4-methylaminoantipyrine (main metabolite)

Metabolites:	4-aminoantipyrine, 4-formylaminoantipyrine, 4-acetylaminoantipyrine
Internal standard:	4-dimethylaminoantipyrine (LC-DAD)
Matrix:	fresh liver (calf, cattle), lyophilised liver, tainted liver, liver of animals from conventional and ecological breeding

The development of an LC-MS/MS method for the determination of metamizole, ramifenazone and phenazone in different matrices by using 4-methylamine-d3 and ramifenazone-d3 as internal standards has been started.

Preparations for a validation according to the matrix-comprehensive in-house validation concept for the determination of metamizole, ramifenazone and phenazone in liver, muscle and plasma of cattle and swine have begun, as well.

3.3 Benzimidazoles (Anthelmintics)

The development of a multi-residue method for screening and confirmatory purposes for all relevant benzimidazoles and levamisole on the basis of LC/MS and LC/MSMS was continued. The target matrices are porcine, bovine, ovine and turkey muscle, liver, kidney and fat.

Analytes examined: albendazole with 2-aminoalbendazolsulfone, albendazolsulfoxide, albendazolsulfone, levamisole, thiabendazole with hydroxythiabendazole, mebendazole with aminomebendazole and hydroxymebendazole, fenbendazole with oxfendazole, oxfendazolsulfone, oxibendazole, flubendazole, parbendazole, triclabendazole with triclabendazolsulfoxide and triclabendazolsulfone, cambendazole, internal standard: nocodazole

The following examinations were carried out:

- Testing of different methods described in literature
- Testing of different polar and non-polar SPE-cartridges: Averaged on all analytes it emerged that diol-cartridges were the most suitable ones
- Optimisation of the solid-phase extraction especially with regard to optimum pH-values and elution solutions
- Preparation of muscle samples (swine)

3.4 Nitroimidazoles

First tests were performed in order to check the applicability of the method for the determination of nitroimidazole in muscle to other matrices. Therefore spiked and incurred liver, retina and plasma samples were investigated. All matrices, so far, have shown promising results. The validation of the method for nitroimidazoles in plasma has been initiated.

3.5 Ractopamine

Due to the recent approval of ractopamine in the USA as food additive for finishing swine, the establishment and validation of a method for ractopamine in muscle was added to the working programme. For this purpose, a sample preparation method submitted by Eli Lilly Laboratories in the framework of approval proceedings was used as a basis. In our laboratory,

some sample preparation steps as well as the detection method were modified. According to the criteria for banned substances (group A) laid down in the revised decision SANCO/1805/2000, the liquid chromatographic separation by means of fluorescence detection figuring in the original method description was replaced by a gas-chromatographic separation by means of mass-spectrometric detection. Ractopamine-d5 is used as internal standard. Two different detection methods (GC/EI-MS and GC/PCI-MS) have to be employed for the confirmation of the analytes, as only two sufficiently intensive and undisturbed diagnostic ions are available per technique.

The validation of this method was finalised and was performed on the basis of the revised criteria of SANCO/1805/2000.

During the validation study it was demonstrated that the method is robust against different matrix conditions, e.g. fresh, lyophilised and tainted porcine muscle.

3.6 Multi-residue method for the determination of beta-agonists including Zilpaterol

Investigations were made for the development of a multi-residue method including as many substances of this group as possible, i. e. both derivatives of the anilin-type and of the phenole-type. Therefore two possible detection techniques were taken into account, firstly the LC/MSMS as a highly sensitive and specific technique and secondly the GC/MS as a widely used technique.

Due to the different physico-chemical properties of the beta-agonists of the phenole- and of the anilin-type, the existing preparation methods cannot simply be taken over for a multi-method. Moreover, with zilpaterol a new structural type was added (benzazepine), whose properties, especially regarding solubility, differ a lot from the other substances.

4 ANIMAL STUDIES

Annex V, chapter 2, section 1 (f))

4.1 Ronidazole (Nitroimidazole)

Intensive stability and homogeneity studies with incurred material were finalised for ronidazole. Numerous analyses of incurred material provided confidence that the extreme differences in the concentration of identical sample material are not exclusively caused by instability, but must be due to the inhomogeneity of the material. In order to confirm this hypothesis an experiment was carried out, during which turkeys were treated with ronidazole. The slaughtering and the sample production were carried out under conditions that excluded as far as possible any decomposition of the analyte in the matrix. The results of these samples reveal an inhomogeneity of the muscle material independent of the muscle type (red [haunch] or white [breast] muscles) and group (superficial or inferior muscles) involved. Therefore the homogenisation and lyophilisation of as much sample material as possible (preferably approx. 500 g muscle) is strongly recommended for routine field laboratories.

Furthermore additional turkeys were treated with dimetridazole and metronidazole in order to investigate the homogeneity in muscle. The investigations have not yet been terminated. Moreover, 28 turkeys were treated with dimetridazole for the investigation of the depletion behaviour of this substance. This substance group was in use for food-producing animals for several decades. The published studies on metabolism and depletion are quite rare and were carried out with analytical techniques not fulfilling modern standards on sensitivity and selectivity. Additionally, the difficulties arising from the inhomogeneity of the substance in matrix were not taken into account. For these reasons, it seemed recommendable to carry out another study taking into account current knowledge.

4.2 Beta-Agonists

The evaluation of the results of the animal study on the accumulation of selected beta-agonists in retina revealed that an additional study on ractopamine in turkey had to be performed. The treatment and slaughtering of animals were carried out. The final measurement results have not yet been completed.

4.3 NSAIDs in milk

In order to investigate the depletion of selected NSAIDs in milk, four dairy cows were treated with vedaprofen, tolfenamic acid, phenylbutazone and carprofen. Each of the first three substances was administered once to one cow. For five consecutive days milk was sampled in the mornings and in the evenings. Carprofen was administered on five consecutive days and the milk was sampled for nine days. In addition blood samples were taken on the first and third day for tolfenamic acid and vedaprofen and on the first and fifth day for carprofen and phenylbutazone. The results showed that vedaprofen and tolfenamic acid cannot be detected in milk. Phenylbutazone was found in a considerably high concentration 10 hours after treatment, which had decreased to below the decision limit 5 days after treatment. During the administration time an accumulation behaviour of carprofen was observed. The concentration had decreased to below the decision limit on the ninth day after treatment.

5 PRODUCTION OF INCURRED SAMPLE MATERIAL

Annex V, chapter 2, section 1 (a, b, g)

Incurred sample material of different species and substances was produced in order to support the development and validation of test methods as well as for quality assurance purposes and proficiency testing.

For these purposes the following animals were treated and some of them also slaughtered in the institute's own facilities:

Species	Number of animals	Substance group	Matrices
turkey	30	Nitroimidazoles	muscle, retina, plasma, liver
turkey	4	Ractopamine	retina
cow	7	tolfenamic acid vedaprofen phenylbutazone carprofen flunixin	milk
cow	1	Metamizole Phenylbutazone ramifenazone/dipyrone	muscle, liver, kidney, plasma
cattle, sheep, horse	3 x 1	Blank	plasma
cattle	4	Blank	plasma
cattle	4	Blank	muscle
pig	4	Blank	plasma
pig	4	Blank	muscle
calf	4	Blank	plasma

Tab. 1: in-house reference material produced

6 CONTINUATION OF THE IMPLEMENTATION OF THE IN-HOUSE VALIDATION CONCEPT

Annex V, chapter 2, section 1 (a, b)

The in-house validation concept was developed at the CRL Berlin and is one of the possibilities to validate in accordance with draft revision SANCO/1805/2000. It is intended to serve as an instrument to harmonise validation procedures, thereby receiving comparable validation parameters, which is still a big gap in European residue control. Moreover it allows a comprehensive, reliable and sensible validation in an efficient way. Since this concept requires demanding calculations of the performance parameters and the production of a specifically designed experiment plan, a project entitled "Development of a software for validation on the basis of the matrix-comprehensive in-house validation concept" financed by the BgVV was started and scientifically and technical supported by the CRL Berlin.

7 QUALITY ASSURANCE AND QUALITY CONTROL

Annex V, chapter 2, section 1 (a, b, c, g)

7.1 Production of in-house reference material

Part of the samples obtained by the production of incurred sample material (cf. Tab. 1) were used to produce in-house reference material for internal quality assurance purposes like quality assurance samples for control charts and method validation purposes. Apart from that, the reference material was also intended to support interested official laboratories in maintaining their quality assurance systems.

7.2 Maintenance of the QM system in accordance with EN 45000 plus

The CRL Berlin continued the maintenance of the QM system in accordance with EN 45000 plus, now EN ISO 17025. It had been accredited under this system by the responsible accreditation authority AKS Hannover in January 2000.

7.3 Proficiency testing

Official residue control laboratories have to prove their competence by regularly participating in proficiency tests organised or recognised by NRLs or CRLs. These proficiency tests are intended to provide an opportunity to check routine test methods and thus to contribute to an objective evaluation of the performance of the participating laboratories.

Proficiency tests are prepared and performed in compliance with the "International Harmonised Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories" (ISO/REMCO N 280), jointly elaborated by ISO, IUPAC and AOAC.

Within the scope of the protocols of the proficiency tests, the participants are free to use the analytical method of their choice and to decide on an adequate number of replicates. It is, however, recommended to predominantly apply the laboratories' routinely applied methods. The participants are asked to give an outline of their sample preparation and measurement parameters in a questionnaire specially designed for this purpose and also to submit a copy of their SOPs or method descriptions for the applied methods.

7.3.1 Beta-agonists

In June 2000 the CRL Berlin had organised a proficiency test for beta-agonists in bovine retina (Beta-agonist Interlaboratory Study 06/2000, BETA_06/00). Only a few laboratories provided their SOPs for closer evaluation.

The submission of the results was scheduled for September 2000 but had to be prolonged until the end of November. Therefore, the evaluation of the results of the Beta-agonist Interlaboratory Study 06/2000 could only be concluded in April 2001. The participants received their z-scores, an overall assessment and an overview of the results compiled in an anonymous form.

Third countries like Norway and the Czech Republic took part in the Proficiency Test, too.

The report was sent to all participating laboratories and to the European Commission.

Twelve National Reference Laboratories participated in the study, among them two from The Netherlands, i. e. eleven of 14 member states submitted results. Three NRLs did not submit

results for various reasons. Four of eleven NRLs (36 %) did not participate successfully. In general that means that 50 % of all NRLs did not demonstrate their proficiency in the analysis of beta-agonists in retina.

Apart from providing a laboratory assessment, this proficiency test also showed that the number of analytes covered by the analytical scopes of the individual laboratories differs a lot. In order to ensure a broad and homogeneous control of this substance group, the performances of the individual laboratories should be equalised in future.

Classical beta-agonists like clenbuterol and brombuterol are covered and analysed satisfactorily by more than two thirds of the laboratories. However, for cimaterol, a beta-agonist which has been well-known for several years and had been implemented into the analytical methods of 60-80 % of the participating laboratories, the overall results were unsatisfactory and should be improved. Furthermore, the situation needs improvement regarding less common analytes like clencyclohexerol and ractopamine.

Since the substance group of beta-agonists includes substances whose chemical structures vary considerably, the corresponding residue-analytical methods have to meet high requirements. Moreover, there have repeatedly been hints suggesting that new derivatives are constantly being synthesised, which involves a danger of new misuse. Thus, the laboratories are confronted with an ever-changing situation. However, the high number of false negative results also for long-known beta-agonists like cimaterol and ractopamine in particular highlights the fact that the European residue control laboratories are not consistently fit to meet this challenge.

7.3.2 Nitroimidazoles

The Interlaboratory study on nitroimidazoles in turkey muscle of June 1999 (NI_06/99) for various reasons showed very insufficient results. Therefore a second interlaboratory study on nitroimidazoles was initiated (NI_03/01). The problems concerning the stability of the analytes and the inhomogeneous distribution of the analytes within the employed matrix “muscle”, which had already shown during the conception of the previous study, were overcome this time by using lyophilised and homogenised material.

The samples were shipped in March 2001. The deadline for the submission of the participants' results was 30 June 2001.

7.4 Participation

The CRL/NRL Berlin participated in a proficiency test on chloramphenicol in muscle organised by the CRL Fougères. Therefore eight samples were investigated. Results have not yet been provided.

8 ORGANISATION OF A WORKSHOP

Annex V, chapter 2, section 1 (i, e)

The CRL Berlin organised a workshop entitled "NSAIDs and validation according to SANCO/1805/2000" in May 2001, which was attended by 11 scientists from the NRLs of the Member States (the representatives of the Greek and the Austrian NRL unfortunately had to cancel their participation). The workshop was intended to contribute to the development and harmonisation of the approach to the residue control of NSAIDs in the Member States.

8.1 Objective

This workshop was aimed at enhancing the harmonisation of the analytical performances of the National Reference Laboratories on a European scale. Since the validation of test methods is of pivotal importance for the reliability of measurement results and therefore for the surveillance of the compliance with legal thresholds, this issue was a central point during the workshop in addition to the presentation of test methods for NSAIDs.

The CRL Berlin presented its interpretation of chapter 6 of SANCO/1805/2000 in form of written working instructions, which were handed out to the participants (see Annex 1 of the Workshop Manual). These working instructions were then applied to a multi-residue method for NSAIDs in plasma by the participants during a practical exercise. For the evaluation of the measurement results and the calculation of the validation parameters an excel sheet, produced by the CRL Berlin, was used.

The workshop was dedicated to the determination and quantification of NSAIDs. It aimed at combining lectures on the analytical, pharmacological and legal aspects of the substance group with practical exercises in the laboratory. The workshop provided the participants with

an opportunity to complete their knowledge, to discuss analytical problems and to have an exchange on practical experiences.

8.2 Course of the workshop

The programme of the workshop, the main topics of the presentations, a list of participants as well as some useful supplementary information are presented in the workshop manual, which was handed out to all participants at the beginning of the workshop.

The main topics covered were:

- Analytics of NSAIDs
- Pharmacological aspects of NSAIDs in food-producing animals
- Practical application of a validation procedure according to SANCO/1805/2000
- Discussion on SANCO Draft
- Participants' contributions on NSAIDs (analytical methods, sampling)

8.3 Evaluation

All participants came from laboratories nominated by Commission Decision 98/536/EC as National Reference Laboratories for substance group B2e. The participants co-operated in a very concentrated manner in the fulfilment of the set tasks. The extent of their practical abilities and their knowledge on NSAIDs varied considerably (cf. Report on Workshop June 2001). The experiences of the CRL Berlin and of the participants with this subject were discussed and the results of the participants' validation study were presented. In general, it can be summarised that the participants accepted the working instructions as a thorough and realistic approach to the validation of test methods and that the instructions clarified some aspects of SANCO/1805/2000. Many laboratories welcomed the provision of the excel sheets produced by the CRL Berlin on the basis of the SANCO/1805/200 requirements because of the easy handling and the harmonised possibility to calculate validation parameters they offer.

In addition, a survey, which had been carried out in advance among the participants on the NSAID methods used in their laboratories, provided an overview of the possibilities existing for the control of NSAIDs.

Ten of the 14 member states answered the questionnaire, including the NRL/CRL Berlin. UK, Spain, Greece and The Netherlands did not provide information on methods for NSAIDs. Five member states investigate more than one substance [Italy (7 different NSAIDs), Austria (4 different NSAIDs) France (Arylproprionic derivatives), Ireland (5 different NSAIDs and Germany (12 different NSAIDs)]. Denmark and Sweden analyse only PBZ. Finland analyses only Flunixin and Flunixin-Hydroxide. Belgium analyses Salicylic Acid. Portugal analyses Tolfenamic acid.

In general it emerged that the analysis of NSAIDs is not very extended and not very well established in Europe. Only three Member States investigate the very important Phenylbutazone, and according to the questionnaire only Germany investigates the very frequently applied Metamizol/Dipyrone. Some of the Member States have only established screening methods, which is not acceptable for National Reference Laboratories. Some of the quoted confirmatory methods use the HPLC-DAD technique, which, according to the "new criteria", will not be sufficient any longer for the confirmation of banned or non-authorised substances like e. g. Phenylbutazone and Ramifenazone.

9 TECHNICAL AND SCIENTIFIC SUPPORT TO NRLS AND THIRD COUNTRIES

Annex V, chapter 2, section 1 (d, f, h, l)

9.1 Analytical Support

During the period covered by this report the CRL Berlin carried out a total of 9 confirmatory analyses of chloramphenicol and beta-agonists.

In particular with respect to confirmatory analyses the CRL Berlin provided technical advice to several NRLs and other official laboratories.

In total 56 analytical methods were shipped to European residue control laboratories and to third countries working in the area of official residue control.

9.2 Standard substances

In accordance with the responsibilities laid down in Council Directive 96/23/EC of 29 April 1996, the CRL Berlin provided 680 shipments of reference standards to the NRLs in the EU Member States and in third countries as well as to official residue control laboratories in Germany (as of 30/06/01). 22 of these substances, especially deuterated internal standards, had been ordered and synthesised on special request of the CRL, but due to the very limited budget for consumables nearly all of these orders and syntheses had to be financed by the BgVV budget.

9.3 Training

The CRL Berlin continued to provide training courses to scientists from EU Member States and third countries. Two one-week-courses were performed providing training in the HPLC-DAD technique and in the determination of NSAIDs in milk and plasma and in GC_MS for the determination of beta-agonists in various matrices.

1. The NRL in Greece responsible for B2e substances requested a training course on the determination of NSAIDs. The training was realised in September 2000. One representative of the laboratory visited the CRL Berlin for one week and was trained in the analysis of acid NSAIDs in plasma by means of HPLC-DAD and in the application of quality assurance measures as established in our laboratory. One scientist and one technician dealt with the trainee during the whole week. The SOP on the determination of NSAIDs by HPLC-MS was provided to her, als well.
2. One of the Argentinian CREHA network laboratories requested a training on beta-agonists. The training was realised in June 2001. One representative of the laboratory visited the CRL Berlin for one week and was trained in the analysis of beta-agonists in urine and retina. Three different methods were demonstrated and carried out by the trainee himself. The produced extracts were analysed by two different analytical techniques (GC-MS and LC-MSMS). Six different method descriptions (SOPs) for anilinic and amphoteric beta-agonists in retina, urine and liver were handed out. In addition, quality assurance measures were demonstrated and explained and English translations of important SOPs e. g. on sample receipt, balances, pipettes and on validation principles according to EU requirements were provided.

9.4 Audits

The CRL Berlin was audited three times during the last contract period.

One audit, carried out by the Canadian Food Inspection Agency (CFIA), took place from 17 to 19 October 2000. The conclusion of the draft report of the CFIA stated that regarding the functions of the CRL Berlin, no deviations from the written SOPs and the quality management system were revealed.

Another audit, carried out by the Food Safety and Inspection Service (FSIS), followed on 31 October 2000. It was a one-day inspection where questions of structure, responsibilities, tasks and the quality management system of the CRL Berlin were discussed and audited. Neither a report nor any conclusions were provided.

A third audit was carried out by the FVO, DG SANCO, in May 2001. It was a one-day audit on 9 May in connection with the mission that took place in Germany from 07/05/01 to 18/05/01 in order to evaluate the control of residues in live animals and animal products. The CRL was audited with respect to the fulfillment of its legal tasks as laid down in Council Directive 96/23/EC, Annex V, and its quality management system. The draft report stated that the laboratory was accredited according to international standards and that the implemented quality management system was of a very high standard in general and especially as regards validation studies of analytical methods. Nevertheless, not all substance/matrix combinations of the list of CRL substances were covered by operational methods yet. However, the Commission services had always been involved in the prioritisation of method developments. All further legal tasks like proficiency tests, workshops, provision of standard substances, production of incurred material and provision of technical and scientific assistance were fulfilled. Nevertheless, the inspection team stated that, in view of the tasks to be fulfilled by the CRL/NRL department, there is still a lack of staff especially as regards technical assistants.

Due to unsolved difficulties and questions regarding the auditing of/visits to member states' laboratories, nor audit/visit was carried out by the CRL Berlin in this contract period. However, the competent Commission service was always involved in dealing with these problems.

9.5 Technical assistance

Approximately 60 inquires from residue control laboratories, ministries and Commission services for technical and scientific assistance were dealt with and answered. Among them there were, e. g., requests for information on the performance of analytical instruments and on the applicability of test methods. In addition, information on the introduction into the European residue control system was provided to third countries like Poland (in the framework of the Phare project), China (Food and Environment Department), China (Ministry of Agriculture) and Lithuania, including oral presentations, discussions and visits to the CRL's laboratories.

10 TECHNICAL AND SCIENTIFIC ASSISTANCE TO THE EUROPEAN COMMISSION

Annex V, chapter 2, section 1 (j)

In the framework of MRL procedures 18 industry methods were sent to the CRL Berlin for the assessment of their applicability for residue control purposes and the fulfillment of EU requirements. The respective reports were sent to the Commission, DG SANCO.

The CRL Berlin was also extensively involved in the discussions and statements as regards the approval of the draft revision of Commission Decisions 93/256/EC and 93/257/EC (SANCO/1805/200).

A survey was started in order to collect information on the applicability and validation parameters of analytical methods used in official residue control. A summary of the NRLs' data on beta-agonists was provided to the Commission.

The CRL director participated in an EC audit mission to Argentina as a "National Expert" (20 November to 01 December 2000).

The CRL director participated in an EC audit mission to Germany as an accompanying person (07 May to 18 May 2001).

Regrettably, only one 4-CRL/Commission meeting was organised during this contract period. It was held in Rome from 29 to 30 May 2001.

A final report on the contract period August 1999 to June 2000 was provided.

A preliminary report on the contract period July 2000 to June 2001 was provided.

Two work plans and two cost estimates were produced and sent to the Commission, covering a one and a two-year contract period, respectively.

The Commission was informed about serious concerns caused by the limited extent of the budget which has not been increased for years, although the costs for staff are increasing permanently due to regular wage rises. This has led to a situation where virtually no share of the fixed budget is available for consumables any longer. No reaction from the Commission services was received .

11 ANNEX

11.1 Publications, Reports and Contributions

1 IT-project proposal on the development of a software for a validation on the basis of the matrix-comprehensive in-house validation concept. 16 August 2000.

2 D. Behrendt, "The European residue control system - contributions of the Community Reference Laboratory Berlin", Microchemical Journal, 67, 2000, 1103 -1107.

3 F. André, K. K. G. De Wash, H. F. De Brabander, S. Impend, L. Van Ginkel, R. Schilt, D. Courtheyn, Y. Bonnaires, P. Fürst, P. Gowik, G. Kennedy, T. Kuhn, J.-P. Morétain and M. Sauer, "Trends in the identification of organic residues and contaminants: a revision of Commission Decision 93/256/EC, accepted.

4 Working Group [André F (chair), Bonnaire Y, De Brabander H, Courthey D, Gowik P, Fürst P, Kennedy G., Kuhn T, Morétain J-P, Sauer M, Schilt R, Ginkel LA van] on Draft of Commission Decision laying down performance criteria for analytical methods to be used for certain substances and residues thereof in live animals and animal products according to Council Directive 96/23/EC (SANCO/1805/2000). Final Draft, October 2000, 50 pp.

5 Reports on the findings made at the three Argentinian laboratories visited during the inspection of the Argentinian residue control in live animals and products thereof from 20 November to 01 December 2000.

6 Final report on a mission to Argentina from 20 November to 01 December 2000. Evaluation of the control of residues in live animals and animal products. DG (SANCO)/1152/2000 - MR final.

7 Draft report on a mission to Germany from 07 May to 18 May 2001. Evaluation of the control of residues in live animals and animal products. DG(SANCO)/3267/2001 - MR draft.

8 Workshop manual May 2001, "NSAIDs and Validation according to SANCO/1805/2000", CRL Berlin, 07-09 May 2001.

9 Report on Workshop "NSAIDs and Validation according to SANCO/1805/2000", CRL Berlin, 07-09 May 2001, 06.06.01.

10 Report on Interlaboratory Study BETA_06/00, CRL Berlin, May 2001.

11 Merging of the reports of the 4 CRLs of the reference periods August 1998 to June 2000.

12 Preliminary report on the activities of the CRL Berlin for the reference period July 2000/June 2001.

13 Cost estimates and work programmes for the forthcoming reference period 01/02 and for a two-year period 01/03.

11.2 Presentations

1. Radeck W., "LC-MSMS in residue analysis of pharmaceutically active substances", 18 May 2001, Leipzig, Germany.

2. Stachel C., "NSAIDs in veterinary medicine", CRL Workshop, 08 May 2001.

3. Radeck W., "Determination of Metamizol metabolites by HPLC-DAD and LC-MS", CRL Workshop, 08 May 2001.

4. Polzer, J., "The validation of methods according to SANCO/1805/2000 – practical application of the regulations presented on the basis of an example", CRL Workshop, 08 May 2001.

5. Fauhl, C., "Experiences gained during the development of a method for the detection of NSAIDs in milk", CRL Workshop, 09 May 2001.

11.3 Staff of the CRL Berlin

Management

P. Gowik	Director of the CRL Berlin (not financed by the EC)
C. Stachel	Deputy Director,
	Head of the division for animal studies, reference material (not financed by the EC)

Analytical Services

W. Radeck	Senior Scientist, Head of HPLC division
J. Polzer	Senior Scientist, Head of GC-Division
	Quality Assurance Officer
C. Fauhl	Scientist, HPLC division
C. Bieber	Technician (85%)
M. Jaber	Technician (66%)
S. Maidhof	Technician (66%)

Administration, Documentation, Translation

M. Jüsgen	Translator

COMMUNITY REFERENCE LABORATORY

for Antimicrobial Residues

SUMMARY

ACTIVITIES OF THE
COMMUNITY REFERENCE LABORATORY AT THE

AGENCE FRANCAISE DE SECURITE SANITAIRE DES ALIMENTS

(A.F.S.S.A.)

Laboratoire d'études et de recherches sur les médicaments vétérinaires et les désinfectants (LERMVD)

CRL-AFSSA-LMV, Fougères

[European Commission Contract Period: July 2000 – June 2001]

P. MARIS

Head of C.R.L.

1 GENERAL

In order to carry out the functions and duties laid down in Council Directive 96/23/EC of 29 April 1996 (Annex V – Chapter 2), the Community granted a financial assistance of EUR 400 000 by a Commission Decision 2000/704/CE of 3 November 2000 for the period 1st July 2000 to 30th June 2001.

In support of this decision a draft of activities programme and a draft budget was sent to D.G. Sanco to be presented to the Standing Veterinary Committee.

CRL-Fougères is concerned by the following residues:

- Antibacterial substances including sulfonamides and quinolones
- Dyes
- Carbadox and olaquindox
- Unauthorized substances included in annex IV to Council Regulation n° 2377/90

The CRL are ongoing these activities by developing new analytical procedures and new analytical technologies, by organizing proficiency tests and workshops, by developing dossiers, by realizing audits etc. Attention has to be drawn to the fact that the contacts with the third countries are increasing and we are starting a network with the Official laboratories.

2 METHODS DEVELOPMENT

The very wide diversity of the antibacterial substances (about 50), the animal species and the matrices and very often the weak means of some NRLs to develop new analytical procedures justify the continuation of this work within the close cooperation of the NRLs.

2.1 Analytical development using H.P.L.C. (High Performance Liquid Chromatography):

- Preliminary studies concerning Aminoglycosides (HPLC)
- Application of the analytical procedure Quinolones to liver, kidney and plasma
- Application of the analytical procedure Macrolides to kidney and milk, and to tylosine + josamycine
- Starting of a work with thiamphenicol, florfenicol and florfenicolamine
- Multiresidues method concerning 4 cephalosporins by HPLC/UV-DAD (cefquinome, cephapirine, desacetylcephapirine and cephalexine – muscle)

2.2 Analytical development using M.S. (Mass spectrometry)

- Development of a confirmatory method for Carbadox + metabolites from pork muscle. The validation step will spend during the next period (LC – MS/MS)

Starting of the development of a confirmatory method for Olaquindox + metabolites

3 OTHER RESEARCH

3.1 ELISA Techniques

- Evaluation and comparison of 2 commercial kits ELISA to detect chloramphenicol from milk.

3.2 MICROBIOLOGICAL Techniques

- Stability studies of antibiotics soaking paper disks usable at short term in an inhibition test in order to evaluate the field laboratories.

3.3 BIOSENSORS

- Detection of 9 penicillins in milk using a biosensor after 2 different treatments: chemical or enzymatic (penicillinase) treatment.
- Writing of 2 protocols concerning the evaluation of sulfamethazine in milk.

3.4 ELECTROCHROMATOGRAPHY and other ANALYTICAL TECHNOLOGIES

- Within the framework of new technologies we are starting a work in close cooperation with Lyon University to better know the utilization of capillary electrophoresis, electrochromatography and micro-H.P.L.C. to detect drug veterinary residues.

4 QUALITY ASSURANCE

Organization of three circular tests

① January 2001: Comparison of the STAR-Milk and Delvotest (14 participants)

② April 2001: Proficiency study for the analysis of Quinolones in pork muscle (17 participants – sending of 10 frozen samples)

③ April 2001: Proficiency study for the analysis of chloramphenicol in pork muscle (LC/MS – LC/MS-MS – GC/MS)

Preparation of a document "Guide for the treatment of samples outside and inside laboratories"

Preparation of a Quality Plan for Organization of the proficiency test

5 AUDIT OF ONE NATIONAL REFERENCE LABORATORY

Two scientists visited National Reference Laboratory Belfast (June 2001)

6 TECHNICAL AND SCIENTIFIC SUPPORT TO DG SANCO NRLS AND THIRD COUNTRIES

- ***Updating*** of the list screening, post-screening and confirmatory analytical methods (June 2001).
- Organization of a ***meeting*** "CRL-NRL" 19 and 20 September 2000 to plan and to coordinate the activities for the next 2 years (24 participants).
- **Training course** "Macrolides and HPLC" (4 sessions in October and November 2000)
- **Meeting of the 4 CRLs** with DG Sanco (Rome, 29 and 30 May 2001).
- **Sending of** a dossier concerning the stability of antibiotic residues in animals and animal products (September 2000).
- **Third countries**: we are starting the putting in place of a network of official laboratories in charge of drug veterinary residues.

Besides these particular activities numerous exchanges have taken place about technical informations, analytical procedures and diverse documents.

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7 ANNEX 1

7.1 Publications, Reports and other Informative Materials

- Dossier "Meeting of the National Reference Laboratories for antimicrobial residues in Food" 19th and 20th September 2000 (Fougères) – 24 participants.

- Dossier: "Stability of active substances" (September 2000).

- Guide for the "Treatment of samples outside and inside laboratories during (June 2001) the storage and preparations".

- Dossier: for the "Meeting Foodsense" concerning the E.C programme launched in June 1994 to demonstrate the applicability of optical sensors for analyses of veterinary drug residues in animal products.

- Reports of 3 students under instructions:
  - Development of an analytical method to detect residues with Biotechnology (April – July 2000)
  - Evaluation and comparison of two commercial kits ELISA to detect chloramphenicol in milk (April – June 2001)
  - Antibiotic residues and micro HPLC (May – June 2001)

- Non offcial list of National Reference Laboratories or Official laboratories outside E.U. but inside Europe (third countries).

- List of the screening, post-screening and confirmatory – analytical methods used in the NRLs of the E.U. countries for antimicrobial residues analysis.

- Report of the audit of the NRL Belfast.

♦ Publications:

- Verdon E., Couëdor P., Laurentie M., Maris P. – L.C. Determination of ampicillin residues in porcine muscle by a multi-penicillin analytical method: a European Collaborative study, sent for publication (april 2001) to J.A.O.A.C. International.
- Gaudin V., Maris P. – Development of a biosensor based immunoassay for screening of chloramphenicol residues in milk. Food and Agricultural Immunology 13 (2), 77-86.
- Gaudin V., Fontaine J., Maris P. – Screening of penicillin residues in milk by a surface plasma resonance – based biosensor assay: comparison of chemical and enzymatic sample pre-treatment. Analytical Chimica Acta 436 (2), 191-198.
- Juhel-Gaugain M., Mc Evoy J., Van Ginkel L. – Measurements for certification of chlortetracycline reference materials within the European Union Standards, Measurements and Testing programme, accepted for publication 9th August 2000 in Frenesius Journal of Analytical Chemistry.

8 ANNEX 2

8.1 Organisational chart and staff of the CRL

(A.F.S.S.A. – Laboratoire d'Etudes et de Recherches sur les Médicaments Vétérinaires et les Désinfectants – FOUGERES)

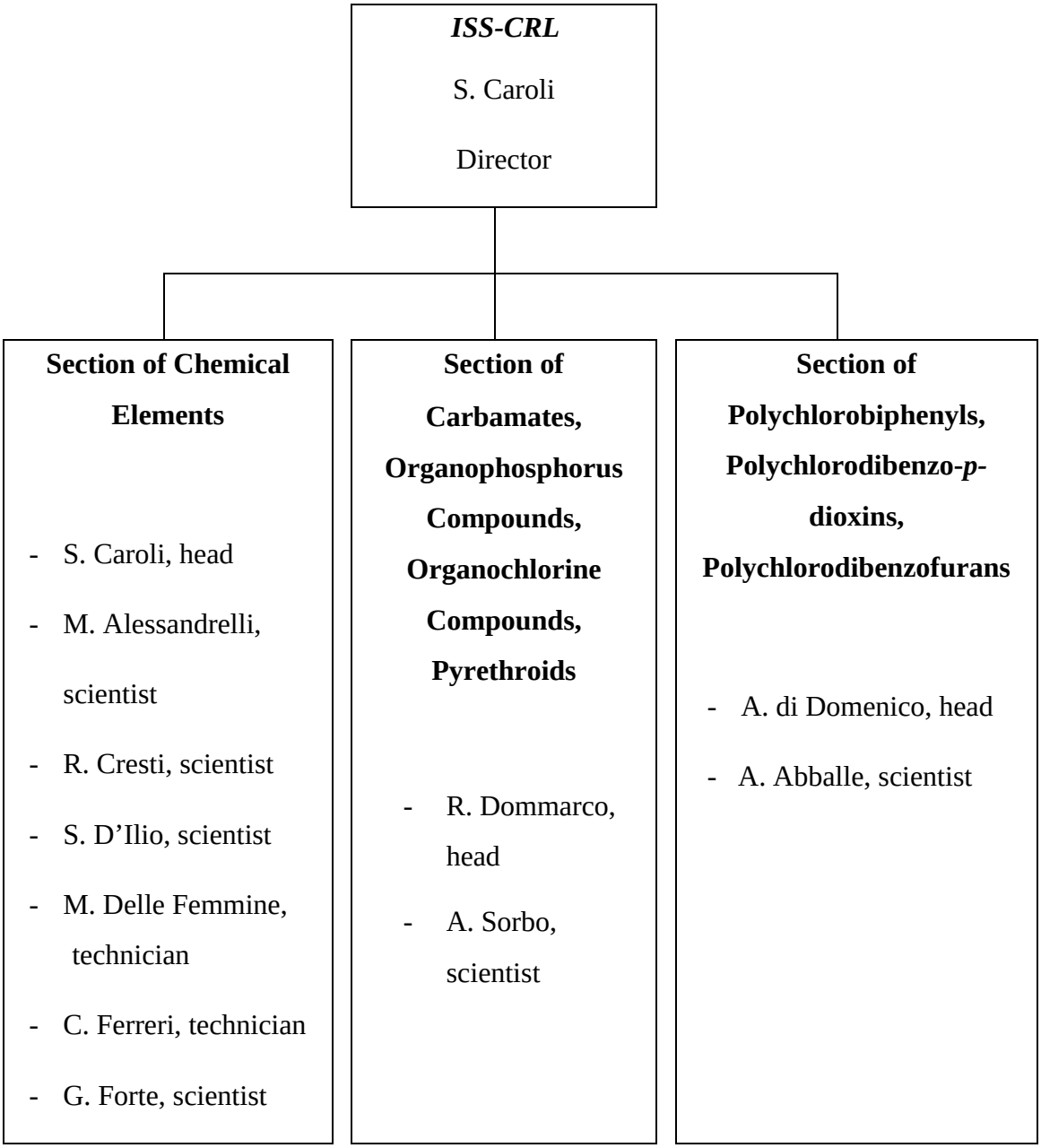
P. Maris,	Head of the C.R.L.
J.P. Abjean,	QA/QC Officer
V. Juban,	QA/QC Officer
B. Anger,	Member of the Unit for analysis of antibacterial substances by H.P.L.C., technician (until January 1999, replaced by J. Blot)
J. Blot,	Member of the Unit for analysis of antibacterial substances by H.P.L.C., technician (since 1st March 1999)
N. Cadieu,	Member of the Unit for microbiological analysis, technician
P. Couëdor,	Member of the Unit for analysis of antibacterial substances by H.P.L.C., technician
R. Fuselier,	Member of the Unit for microbiological analysis, scientist
V. Gaudin,	Member of the Unit for immunological analysis, Scientist
D. Hurtaud-Pessel,	Member of the Unit analysis of antibacterial substances by M.S., Scientist
M. Juhel-Gaugain,	Member of the Unit analysis of antibacterial substances by H.P.L.C., Scientist
A. Rault,	Member of the Unit for antibacterial substances, technician
E. Verdon,	Member of the Unit for analysis of antibacterial substances by H.P.L.C., scientist
J.C. Yorke,	Member of the Unit for analysis of antibacterial substances by H.P.L.C., scientist
C. Marcault,	Secretary
C. Gervis,	Secretary

Annual Report on the Activities of the ISS-CRL

CRL-ISS, Rome

July 1, 2000 – June 30, 2001

1 ORGANIZATIONAL CHART OF THE ISS-CRL



2 GENERAL ASPECTS

On the basis of the outcome of the activities of the past reference period and in fulfillment of the plans approved by DG SANCO for the period July 1, 2000 – June 30 2001, the Rome CRL has further discharged its duties in compliance with the mandate assigned by the Council Directive 96/23 of April 29, 1996, as described below.

3 PROFICIENCY TESTING

The sixth interlaboratorial trial on quality assurance of trace element determinations in matrices of animal origin was undertaken and completed. This series of proficiency tests now replaces the previous Phase 1.2a of the ongoing Quality Assurance Programme for the determination of trace elements in complex matrices and is intended to constantly monitor the performance of National Reference Laboratories (NRLs) for residues in the analysis of solutions containing As, Cd, Cr, Cu, Fe, Hg, Pb and Zn, enriched with salts to simulate the acidic digestion of real meat samples.

The results of the recently undertaken proficiency test for the determination of trace elements were fully evaluated and discussed during the Workshop held in Rome, May 28-29, 2001. Quality of measurements was generally satisfactory for all elements with the exception of Hg, for which some laboratories found values much lower than the target value. This can be probably traced back to the fact that the solutions to be analyzed had been stored too long before being processed. Due consideration was given to this aspect and during the Workshop it was decided that analyses are to be performed within two weeks from the receipt of samples. Moreover, during the meeting it was also agreed that the frequency of the tests should be increased. From now on, therefore, such exercises will be repeated on a quarterly basis in order to better monitor the performances of NRLs over time. In order to make the participation of NRLs in such exercises still compatible with their daily workload, the number of elements to be determined decreases from ten to six per each test in such a way

that As, Cd, Hg and Pb will be always present and either Cr+Zn or Cu+Fe will be added to the above four elements.

In conformity to the plans previously established, a proficiency test for the determination of trace elements in honey was also undertaken. Honey, in fact, is one of the natural foodstuffs of animal origin largely used for human consumption thanks to its nutritional properties. In addition to this, over the past few decades, honey was also proposed as a biological indicator of environmental pollution. Potentially hazardous contaminants deposited on flowers and absorbed on soil and water, toxic metals included, are transported by the bees to the hive.

Taking advantage of the ongoing certification of *Acacia* and *Eucalyptus* honey for some trace elements (see next section), the candidate CRMs prepared by the Institute for Reference Materials and Measurements of the Joint Research Centre, European Commission (EC-JRC-IRMM) in the frame of the existing MoU were partly exploited to perform a proficiency test in this matrix. Two sets of approximately 200 ampoules per each type of honey were used to this end. Six ampoules for each of the two types of honey were sent to the participants who were asked to quantify As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se and Zn (mandatory set) and possibly Mn, Sn and V (optional set). No digestion of the test material was recommended.

The analysis of the concentrated aqueous solutions of honey turned out to actually cause some problems when performed by Atomic Absorption Spectrometry techniques because of the lack of measurement reproducibility and formation of both solid particles and foam. In spite of this, the outcome of the exercise was in general satisfactory. For most elements the data obtained for each analyte were rather consistent and useful information could be reached as regards the possibility of their certification (*e.g.*, for Cu, Fe, Mn, Ni and Zn). Also for As, Cd, even if they are present at very low levels, and for Pb, which is prone to the external contamination, certification might be achieved. In the case of Sn and V, only two sets of data for each element were supplied. Still, these values can be of some help in view of their possible future certification. Furthermore, the data obtained for Se were so much scattered that no useful information could be obtained, while those for Hg were all below the detection power of the techniques used for its determination. Finally, the determination of Cr turned out to be strongly influenced by matrix effects.

4 PREPARATION OF NEW CERTIFIED REFERENCE MATERIALS (CRMS)

The lack of Certified Reference Materials (CRMs) prompted the ISS-CRL to launch two separate certification projects. The preliminary phases of these two projects were already described in the Annual Report of the ISS-CRL for the period July 1, 1999 - June 30, 2000. In consideration of the unsuccessful outcome of the pilot study (several hundreds of ampoules containing aqueous solutions of *Acacia* and *Robinia* honey were spoiled by microbial growth and had to be discarded), collection of honey for the preparation of two candidate reference materials was started again. Thus, ten kilograms each of two different types of honey, namely *Acacia* (liquid and viscous) and *Eucalyptus* (solid and viscous) were sampled. The collection of the *Acacia* honey was performed in May 2000 in the Lombardy region (area of Como), whereas that of the *Eucalyptus* honey took place in July 1999 in the Tuscany region (area of Grosseto). In this way, the two planned CRMs can reasonably cover a variety of needs. In the first place they will be a further means of harmonization of the performance of NRLs for the quantification of trace elements in honey. Quality control schemes can be thus expediently set up that will assist the NRLs in their daily work. The preliminary work to produce the materials candidate for the certification process was again undertaken by the EC-JRC-IRMM. Approximately five thousands of glass ampoules containing ca. 3.6 g of aqueous solutions of *Acacia* and *Eucalyptus* honey (with 20 % and 30 % high purity water, respectively) were prepared and sealed under inert gas (argon). In order to avoid any microbial growth, all ampoules were ?-irradiated (dose 25 kGy) by Gammaster B.V. (The Netherlands). The candidate CRMs featured high homogeneity as tested at the EC-JRC-IRMM prior to ampouling. This is quite understandable because aqueous solutions of honey are dealt with. In fact, once exogenous solid particles were removed from the bulk solutions, these were kept under continuous stirring and ampouled. The mass contained in each ampoule in no cases need filtration upon opening. Random tests conducted on several vials revealed no significant variations in the concentrations of the elements to be certified. Short-term tests pointed also to sufficient stability, although full assessment of this property can be achieved only with long-term tests. Further evaluation of the homogeneity and stability of the two CRMs is still underway. In conclusion, the experimental data obtained to date are supportive of the fact that the approach chosen for pretreating the raw materials can be effective to achieve satisfactorily homogeneous and stable end products. For almost all elements, concentrations in the two types of honey differed significantly. This fact can be ascribed to differences in the botanical

species from which honey was produced, geochemical composition of soil and possibly local environmental pollution. So far, no specific regulations have been issued for trace elements in honey. Even the Codex Alimentarius simply prescribes that "Honey shall be free from heavy metals in amounts which may represent a hazard to human health". The average values obtained for the above elements by means of Inductively Coupled Plasma-based techniques are as follows

(in µg kg-1 ± SD): *Acacia* honey, As, 1.10±0.20; Cd, 0.328±0.035; Cr, 1.90±0.22; Cu, 67.0±5; Fe, 215±30; Hg, < 0.75; Mn, 82.1±6.2; Ni, 21.0±3.0; Pb, 2.30±0.25; Se, 9.10±1.2; Zn, 167±22; *Eucalyptus* honey, As, 5.99±0.10; Cd, 0.592±0.074; Cr, 1.50±0.07; Cu, 219±24; Fe, 1008±114; Hg, < 0.75; Mn, 1009±51; Ni, 11.3±1.5; Pb, 5.00±0.40; Se, 5.60±0.91; Zn, 791±91.

It is possible to anticipate that by the end of year 2001 the certification of the two honey-based CRMs can be successfully completed. These projects will be conducted by the EC-JRC-IRMM on behalf of the ISS-CRL.

5 METHOD DEVELOPMENT

5.1 Trace elements

Speciation of chemical elements has become an investigative tool of high potential to better assess the risk posed to humans by inorganic contaminants in food. This analytical approach was applied to the chemical speciation of As in food and biological material in general. It is well known nowadays that organoarsenical compounds are less toxic than the inorganic ones. Arsenobetaine (AsBet) and Arsenocholine (AsChol), as final metabolic products, are well tolerated by living organisms. Marine organisms are considered to be among the greatest bioaccumulators of As, due to the tendency shown by this element to replace N or P in several compounds. On the other hand, poultry and cattle are often fed with mixed feed, including fish-based meals. In consideration of the above, a study was undertaken aimed at identifying and quantifying some arsenical species in feedstuff and foodstuff, namely, As (III), As (V), Monomethylarsonic acid (MMAA), Dimethylarsinic acid (DMAA), AsBet and AsChol. The

procedure adopted is rather simple: samples are ground in an agate mortar until a fine and homogeneous powder is obtained. Extraction then follows by employing a patented device (Wertex, FKV, Italy). Samples are weighed in the Wertex unit itself and placed into the extraction vessel. Subsequently, 25 ml of a mixture of methanol-water (1:1 v/v) are added inside and about 20 ml of the same solution outside the glass holder of the extraction unit. In order to avoid the degradation of the arsenical species of interest, the extraction programme must be set up so as to preserve their integrity, *e.g.*, a temperature ramp from 20 to 50 °C in 5 min, then 20 min at 50 °C and finally 20 min of ventilation (the increase in power is gauged by the ATC-FO advanced fiber optic for the automatic temperature control). The entire cycle is repeated three times.

Once the extraction is completed the Wertex unit is placed in the FiltEX module for performing filtration. The solution contained in the extraction vessel is poured into the Wertex unit and vacuum-filtered in a few minutes. The evaporation step is carried out in the TURBOVAP work station using 200-ml glass tubes in a water bath at 60 °C (generally with this mixture a minimum period of time of about 15 min is required). An air stream speeds up this step. In fact, a "helical flow" of air is created by a stream of inert gas (Ar) blown into each sample tube; this causes a vortexing action which ensues in better homogeneity of the solutions and continuous rinsing of the tube walls. The solvent vapors are removed by an exhaust fan.

The solution remaining in the evaporation tubes (no more than 500 µl) is diluted with high purity deionized water and injected into the HPLC-Q-ICP-MS system. In order to achieve an optimum separation of the different As forms, the pH of the mobile phase (0.03 M NaHCO3) was adjusted to 9.

Preliminary results shows that: i) is not necessary to repeat the extraction cycle three times because passing from the first cycle to the third one no further enrichment in analytes occurs in the solution; ii) the extraction procedure described above does not seem not to be fit for purpose because the recovery yield is poor (never more than 60 %); iii) optimization of the two steps above is mandatory.

5.2 Organic substances

Substitutes of urea compounds are widely used as herbicidal agents, such as the phenylurea and sulphonylurea derivatives. These compounds are generally stable in aqueous systems and will not easily undergo hydrolysis except under extreme acid or basic conditions. For this reason they can contaminate surface water used for crop irrigation and increase the possibility of unacceptable residues in drinking water.

An analytical procedure has been developed for the simultaneous determination of some sulphonylurea compounds (Benzthiazuron, Chlorsulfuron, Diuron, Linuron, Chlorbromuron, Primsulfuron, Diflubenzuron, Neburon and Triflumuron) in drinking water. The extraction procedure from drinking water has been performed by Solid Phase Extraction (SPE) with a C18 cartridge. The extracts have been analyzed by High Performance Liquid Chromatography (HPLC) with Diode Array Detector (DAD). The quantification limits (from 0.001 to 0.01 µg l-1) of these compounds were below the European regulatory limit of 0.1 µg l-1. This experimental approach shows much promise also for other pesticides.

6 TECHNICAL AND SCIENTIFIC SUPPORT TO NRLS AND THIRD COUNTRIES

The Handbook of Analytical Methods for Trace Elements as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996) was revised (updated edition, May 2001) and circulated among all nRLs for comments and additions. Other handbooks for organic compounds were undertaken, devoted respectively to organochlorine compounds, carbamates, pyrethroids, organophosphate and polychlorobiphenyl compounds, polychlorodibenzo-*p*-dioxins, polychloro-dibenzofurans. These are being implemented with the information supplied by the NRLs.

All handbooks present the same standard format. The first Section is devoted to the type of samples more frequently analyzed (*e.g.*, meat, liver, kidney, fish, *etc.*). The Section on Samples throughput, in turn, focuses on the average number of samples analyzed every year by each laboratory. The Section Procedures takes into full account sampling, storage,

subsampling and pretreatment criteria adopted by each NRL (Subsection 3.1.a), whereas the type of analytical instrumentation employed and the working conditions (instrumental settings, calibration mode, *etc*.) are reported in Subsections 3.1.b and 3.1.c, respectively.

The Quality control part provides the details of the quality control/quality assurance schemes adopted, with particular emphasis on the use of CRMs. The Section Difficulties and limitations focuses on all the problematic aspects encountered in the determination of the residues of interest. Last but not the least, in Comments and remarks the NRLs are requested to give suggestions about possible improvements of the Handbooks.

In the case of organic compounds, two specific Subsections, namely Extraction and Clean-up, had added in the Section Procedures, between the Subsections Sample Pretreatment and Analytical Instrumentation. They contain information respectively on the methodological approaches used to extract the organic compounds from the relevant matrices and on their further purification.

7 PARTICIPATION IN CONFERENCES, WORKSHOPS AND COURSES

1. *Users' Meeting of Mass Spectrometry,* Certosa di Pontignano (Siena, Italy), September 20-22, 2000 (one communication).

2. *1st International IUPAC Symposium, Trace Elements in Food*, Warsaw (Poland), October 9-11, 2000 (one communication).

3. *National Training Course for GLP Inspectors (D.L.vo nr. 120/92),* Rome (Italy), November 13-14, 2000 (organization and conduct of the course).

4. *Sixth Rio Symposium on Atomic Spectrometry*, Concepción (Cile), December 3-9, 2000 (five communications).

5. *Second International Conference on Trace Element Speciation in Biomedical, Nutritional and Environmental Sciences*, Munich-Neuherberg (Germany), May 7-10, 2001 (chair and one communication).

6. *2nd International Symposium of Pesticides in Food and the Environment in Mediterranean Countries*, Valencia (Spain), May 9-12, 2001 (one communication).

7. *Conference on the New Good Laboratory Practice - Priority, Problems, Perspectives*, Rome (Italy), May 14-15, 2001 (organization, chair and plenary lecture).

8. *Workshop on the Sixth Proficiency Test on Quality Assurance of Trace Element Determinations in Matrices of Animal Origin*, Rome (Italy), May 28-29, 2001 (organization and conduct of the workshop).

8 PUBLICATIONS

1. S. Caroli, G. Forte, M. Alessandrelli, R. Cresti, S. D'Ilio, M. Spagnoli, Gy. Záray, Trace Elements in Bovine Muscle: an Ongoing Project for a New Certified Reference Material, *Microchem. J*., 67 (2000), 235-243.

2. S. Caroli, G. Forte, M. Alessandrelli, R. Cresti, S. D'Ilio, M. Spagnoli, J. Pauwels, G. N. Kramer, A Pilot Study for the Production of a Certified Reference Material for Trace Elements in Honey, *Microchem. J*., 67 (2000), 227-233.

3. S. Caroli, G. Forte, M. Alessandrelli, R. Cresti, S. D'Ilio, M. Spagnoli, The Handbook for Analytical Methods for Trace Elements as Adopted by the National Reference Laboratories for Residue, *Microchem. J*., 67 (2000), 381-384.

4. S. Caroli, G. Forte, M. Alessandrelli, R. Cresti, S. D'Ilio, M. Spagnoli, F. Chiodo, The Cardbox/Cardview Database for Residues in Live Animals and Their Products, *Microchem. J*., 67 (2000), 375-379.

5. S. Caroli, S. D'Ilio, G. Forte, M. Alessandrelli, R. Cresti, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Trace Elements as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

6. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Polychlorobiphenyl Compounds as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

7. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Organochlorine Compounds as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

8. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Carbamates as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

9. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Pyrethroids as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

10. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Organophpsphates Compounds as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

11. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Polychlorodibenzofurans as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

12. S. Caroli, R. Cresti, S. D'Ilio, G. Forte, M. Alessandrelli, M. Delle Femmine, C. Ferreri, Handbook of Analytical Methods for Polychlorodibenzo-p-dioxins as Adopted by National Reference Laboratories for Residues of the European Union (Directive 95/23/EC of April 29, 1996), *ISS Internal Report*, May 2001.

13. G. Forte, S. D'Ilio, S. Caroli, Honey as a Candidate Reference Material for Trace Elements, *J. AOAC Intern*., 2001 (in press).

9 ANNEX 1

9.1 Minutes of the Meeting of Experts from NRLs on the Sixth Proficiency Test on QualityAssurance of Trace Element Determinations in Matrices of Animal Origin

28-29 May 2001

Community Reference Laboratory Staff

S. Caroli, Director

R. Dommarco, Deputy Director

A. Abballe

M. Alessandrelli

R. Cresti

M. Delle Femmine

C. Ferreri

G. Forte

A. Sorbo

Representatives of National Reference Laboratories

G. Mayerhofer, Federal Control Institute for Animal Infections and Diseases, Mödling, Austria

E. Larsen, Danish Veterinary and Food Administration, Søborg, Denmark

E. - R. Venäläinen, National Veterinary and Food Research Institute, Helsinki, Finland

L. Böhm, Staatliches Veterinär- und Lebensmitteluntersuchungsamt, Potsdam, Germany

S. Samartzi, Institute of Biochemistry, Toxicology and Nourishment of Animals, Athens, Greece

J. Murphy, Central Meat Control Laboratory, Dublin, Ireland

M. Baldini, Istituto Superiore di Sanità, Rome, Italy

F. Cubadda, National Reference Laboratory, Istituto Superiore di Sanità, Rome, Italy

T. Oliver, Agriculture and Food Science Centre, Belfast, Northern Ireland

M. da Luz Ferreira, Laboratório Nacional de Investigaçào Veterinària, Lisboa, Portugal

I. M. de La Hinojosa, Laboratorio Arbitral, Madrid, Spain

L. Jorhem, National Food Administration, Uppsala, Sweden

R. Ritsema, Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven, The Netherlands

Representative of the European Commission

P. Robouch, Institute of Reference Materials and Measurements of the Joint Research Centre, European Commission (EU-JRC-IRMM), Geel, Belgium

Representatives of Other Laboratories

I. Ipolyi, University of Horticulture, Food Industry and Biochemistry, Budapest, Hungary

M. C. Abete, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

G. Binato, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

E. Ferretti, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia, Brescia, Italy

Excused

W. Van Rillaer, Institute of Hygiene and Epidemiology, Brussels, Belgium

T. Guerin, Agence Française de Sécurité Sanitaire des Aliments, Paris, France

A. Bortoli, Azienda Regionale Prevenzione ed Ambiente del Veneto, Mestre, Italy

G. Chessa, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy

R. Cozzani, Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Rome, Italy

S. D'Ilio, Community Reference Laboratory, Istituto Superiore di Sanità, Rome, Italy

P. Fonti, Centro Studi Ambientali, Rimini, Italy

M. Spartera, Azienda Sanitaria Locale Taranto 1, Taranto, Italy

P. Trentini, Agenzia regionale Prevenzione Ambiente dell'Emilia Romagna, Ferrara, Italy

T. Waaler, National Veterinary Institute, Oslo, Norway

H. Crews, Ministry of Agriculture Fisheries and Food, Central Science Laboratory, Norwich, United Kingdom

Agenda

Monday, 28 May 2001, 10:00 am – 5:00 pm

1. Welcome to participants
2. Adoption of the Agenda
3. General information of interest to the NRLs for residues
4. Discussion on the results of the proficiency test for the determination of trace elements in synthetic aqueous solutions

Tuesday, 29 May 2001, 10.00 am – 5.00 pm

5. Discussion on the results of the proficiency test for the determination of trace elements in honey solutions
6. Future proficiency testing programmes
7. Updating of the Handbook of Analytical Methods for Trace Elements in use at NRLs
8. Any other business
9. Closure of the meeting

Monday, 28 May 2001

Agenda item 1: Welcome to participants

The Chairman, Prof. Caroli, welcomed the participants and summarized the scope and goals of the Sixth Interlaboratorial Trial on Quality Assurance of Trace Element Determinations in Matrices of Animal Origin. Each participant was then asked to introduce herself/himself. Prof.

Caroli drew the attention of the audience on the organizational problems encountered that delayed the formal approval of this meeting by the Istituto Superiore di Sanità (ISS). He also announced that after the conclusion of the Workshop, a coordination meeting of the four CRLs was to be held at the ISS in order to discuss the future developments of their activities. The content of the folder given to each participant was then illustrated.

Agenda item 2: Adoption of the Agenda

The various items of the agenda were briefly illustrated by the Chairman. The agenda was unanimously approved. The minutes of the past meeting of the National Reference Laboratories (NRLs) for trace elements (Rome, 12-13 June 2000) were approved with no changes.

Agenda item 3: General information of interest to the NRLs for residues

Prof. Caroli gave some information about the introduction of the new unit of the ISS-CRL that was set up to deal particularly with polychlorodibenzo-p-dioxins and polychlorodibenzofurans. This unit has been entrusted to the section of Comparative Toxicology and Ecotoxicology, headed by Dr. A. di Domenico Department. The activities of the ISS-CRL in favour of Third Countries especially those candidates to become Members States of the European Union, were also briefly summarized. As an example of this commitment, the recently renewed (April 2001) bilateral agreement for technical and scientific cooperation between Italy and Poland envisages also assistance to this latter country by the ISS-CRL to encourages the adoption of criteria harmonized with those of the EU in the management of residues in animal products. To this end, next October, in Warsaw a basic training course for scientists of potentially interested public institutions will be held by two representatives of the ISS-CRL.

Agenda item 4: Discussion on the results of the proficiency test for the determination of trace elements in synthetic aqueous solutions

Dr. Cresti (ISS-CRL) summarized the goal of the sixth exercise (determination of As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se and Zn in solutions simulating the digest of real meat samples) and

illustrated the procedure adopted for the statistical treatment of data supplied by participants. The discussion of the results followed. As done in the previous exercise for the evaluation of the performance of laboratories, also this time the Z-score approach was resorted to.

For values of $|Z| \leq 2$ the performance of the laboratory was considered acceptable. Moreover, the assessment of long-term trends was carried out by two different approaches. To this end the analytical results were evaluated in terms of percentage of unacceptable data, *i.e.* exceeding by more than ± 10 % the target value. Moreover, the Z-scores were combined by using the Rescaled Sum approach in order to obtain more information on the trend shown by each participants over time.

For each element the problems encountered by each laboratory during the analysis were highlighted. Particular emphasis was devoted to the discussion about the use of Certified Reference Materials (CRMs) in carrying out the determination.. For the next proficiency test, the participants agreed that the use of CRMs, although recommended, is not mandatory, this decision being only up to the laboratory.

As regards the Cu determination, Dr. Mayerhofer (Austria) raised the problem of the possible contamination of the standard solutions. Prof. Caroli deemed appropriate to inquire what procedures are adopted by the participating laboratories to prepare calibrants and whether their stability is checked regularly. At the end of the discussion, it clearly resulted that the majority of laboratories make regular use of standard solutions, although no long-term stability tests of calibrants is made.

Serious problems were also found by most participants during the quantification of Hg. It was in fact complained that the stability of this element in solution was insufficient to perform accurate analyses. In this respect, the Chairman suggested to carry out the determination as soon as the samples arrive to the laboratory in order to minimize any stability changes.

At the end of the discussion, the majority of participants were in favour of making of this exercise a permanent part of all future proficiency tests for trace element determination in matrices of animal origin.

To further enhance the benefits of such proficiency tests, the Chairman proposed to modify the present structure of this exercise, *i.e.* to make it quarterly, rather than annual, in each

round only solutions unknown to participants containing six elements out of ten, will be analyzed. Solutions with concentrations known to participants will no longer be supplied. The timing of this exercise implies the samples should be analyzed within two weeks from the receipt and the results should be submitted to the ISS-CRL within the two following weeks. The assessment of the ISS-CRL will be circulated to NRLs.

Finally, the Chairman went around the table to know which laboratories were recently appointed as NRL by the Member States, as prescribe by the Decision Commission 98/536/EC of 3 September, 1998. Except Dr. Mayerhofer (Austria), Dr. Murphy (Ireland) and Dr. Venäläinen (Finland), the other participants replied that they had no official information on the confirmation of their designation.

Tuesday, 29 May 2001

Agenda item 5: Discussion on the results of the proficiency test for the determination of trace elements in honey

The Chairman briefly summarized the process that led to the production of the two new stocks of ampoules containing the aqueous honey solutions. Ten kilograms each of two different types of Italian honey, *i.e.* Eucalyptus (solid and viscous) and Acacia (liquid and viscous) were newly collected. All the pretreatment necessary to produce the materials candidate for the certification process were performed at the Institute of Reference Materials and Measurements of the Joint Research Centre, European Commission (EU-JRC-IRMM). 2176 of glass ampoules for Acacia honey and 2037 of glass ampoules for Eucalyptus honey, each containing approximately 3.6 g of an aqueous solution of Acacia or Eucalyptus honey (with 20 % and 30 % high purity water, respectively) were prepared and sealed under inert gas (argon). This procedure was developed in order to obtain dense solutions with an acceptable degree of homogeneity. To avoid any microbial growth and internal pressure, as it happened in the pilot study all the ampoules were γ-irradiated at a dose 25 kGy by Gammaster B.V. (The Netherlands). Six ampoules of each at the two types of honey were sent to all the participants in the proficiency test. Laboratories were asked to quantify As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se and Zn (compulsory elements) and Mn, Sn and V (optional elements). Each laboratory was requested to submit only one result per element per ampoule for a total of six results for each element and type of honey. The Chairman then gave the floor to Dr. Robouch (EC-JRC-

IRMM, Belgium), who took over responsibility of the certification project for the two honey-based candidate materials. Dr. Robouch explained in detail how the data submitted by laboratories were evaluated. For each set of type of honey, two bar graphs and one Youden plot were produced. The first set of bar graphs give the means of the six values and their standard deviation, while the second set of bar graphs present all the individual values submitted by each laboratory, per element and honey type. The Youden plots, in turn, show the relationship between the mean values per element obtained by each laboratory in the two types f honey, in order to detect possible systematic biases.

It was then decided to go around the table to ascertain what kind of sample pretreatment was adopted prior to the determination of trace elements in honey. All participants had been requested to simply dilute (no digestion) the honey samples for the analyses whether possible. This was, in reality, unfeasible in several cases, especially for Atomic Absorption Spectrometry (AAS).

In fact, in the case of analysis of diluted samples, most of the AAS users reported that: i) the methodology applied for Cd and Pb was irreproducible (Austria and Spain); ii) the pirolysis produced solid particle in the furnace (Greece); iii) foam formed in the reaction cell during the analysis of Hg (Padova); iv) solid suspended particles were observed when HCl was added in the case of Se determination (Finland and Greece). In addition to this, a few laboratories complained that the ampoule content was insufficient to quantify As, Hg and Se (Greece).

Dr. Böhm (Germany) and Dr. Oliver (Northern Ireland) specified that no particular problems occurred, but for some elements data were very low and not so far from the detection limit. Moreover, Dr. Oliver (Northern Ireland) did not dilute the honey samples; rather, these were digested. Dr. Cubadda (Italy) had problems with Cd and Se determinations in the case of diluted samples. Moreover, he supplied data also for digested samples and stated that, in his view, digestion was the best approach for this kind of material, this also implying less dilution of samples. In both cases the standard addition method for calibration was recommended.

Due consideration being given to these remarks, it was decided that plain dilution of the aqueous solutions of honey cannot be imposed because it might be unsuitable for some of the techniques employed. This means that laboratories will be free to use the methodological approach which ensure the best performance of their instrumentation.

Another problem was related to the quantification of Cd and Pb: in fact, some participants found to high concentration values, probably because of contamination phenomena. Dr. Larsen (Denmark), Dr. Samartzi (Greece), Dr. Jorhem (Sweden) and Dr. Ritsema (The Netherlands) asked whether this could be related to the deposition of those elements on the external surface of the ampoules from exogenous sources or whether they might be released from the vial glass. Dr. Robouch replied that studies, carried out at several temperatures on the glass employed for the vials, reveled that apparently no release of elements from that material took place. Anyway, the Chairman suggested to reconsider the possibility of using plastic containers for this kind of solutions.

In general, the proficiency test for the determination of trace elements in aqueous solutions in Acacia and Eucalyptus honey, was satisfactory enough after withdrawal of a few sets of data: for which clear sources of errors could be identify, *i.e.* those of Dr. Ipolyi (Hungary) (all data for As, Pb and Se due to the high background caused by the organic matrix), of Dr. Binato (Padova, Italy) (the sixth value of each element for both honey samples) and of Dr. Ferretti (Brescia, Italy) (the Zn because of malfunctioning of the lamp).

In particular, the exercise was quite acceptable also in the case of As and Cd, given the very low level of these two elements in the honey solutions (in the range below ng g^{-1}).

In turn, data for Cr were affected, in many cases and for both types of honey, by a relatively high standard deviation. It was also noted that analyses performed by Q-ICP-MS led to data relatively higher than those obtained by HR-ICP-MS and DRC-ICP-MS.

Most of the data obtained for Hg were below the detection limit of the analytical technique used.

Also the results obtained for Pb were affected by relatively high standard deviations, probably because of the random contamination that can easily occurr.

As regards Se, just a few laboratories supplied data. They were so diverging from each other that no useful information could be obtained.

In the case of Sn and V only two sets of data were supplied in rather close agreement with each other.

Dr. Robouch explained that the values submitted will be used, where possible, to produce reference or consensus values. This information will be greatly useful also in view of the certification project that will be probably concluded by the end of the current year.

Agenda item 6: Future proficiency testing programmes

As already mentioned under point 4 above, the number of proficiency tests per year should be substantially increased because with only one it is not possible to efficiently monitor the performance of each laboratory along twelve months. The Chairman thus suggested to have four ring tests per year based on unknown solutions containing subsets of the elements of interest in various combinations and at various concentrations.

In this connection, Dr. Jorhem (Sweden) observed that it could be more important to focus future proficiency tests on elements dealt with in legal acts (As, Cd, Hg and Pb). Moreover, it is also important to take into account the matrices where the contaminants have to be quantified. In fact, Dr. Jorhem informed that his laboratory is accredited for food analysis in general. Dr. Samartzi (Greece) stated, on the other hand, that her laboratory is accredited only for fish analysis.

In light of these comments, the Chairman asked participants to indicate what are the matrices routinely analyzed in their laboratory and the elements normally quantified. This updating of the tasks normally carried out by the NRLs might in fact be exploited to the composition of the synthetic solutions used for the proficiency tests.

Dr. De La Hinojosa (Spain) and Dr. Da Luz Ferreira (Portugal) declared they mostly deal with quantification of As, Cd, Hg and Pb in meat and liver. Dr. Ferretti (Brescia, Italy) determined Cd, Hg and Pb in meat and fish and Cu, Mn and Zn in animal feed.

Dr. Binato (Padova, Italy) was in favour of preparing synthetic solutions with all the ten elements taken into account so far and then let the laboratory free to quantify what they wish. Dr. Ipolyi (Hungary) and Dr. Cubadda (Italy) normally determine food contaminants. Also Dr. Böhm (Germany), Dr. Murphy (Ireland) and Dr. Oliver (Northern Ireland) quantify As, Cd, Hg and Pb in meat and fish. Dr. Abete (Torino, Italy) usually determine As, Cd, Cr, Hg and Pb in meat and liver and Cu, Fe and Zn in foodstuffs. Dr. Ritsema (The Netherlands) several

elements in various matrices. Dr. Samartzi (Greece) stated that the elements more frequently determined are Cd and Pb in meat and liver and Cd, Hg and Pb in fish.

At the end of the discussion on this issue, it was decided that: i) is not necessary to change the composition of the synthetic solutions; ii) the proficiency test will be repeated four times per year; iii) each laboratory will receive one solution with six elements (out of ten) at unknown concentrations; iv) the elements As, Cd, Hg and Pb will be present in all solutions and the concentration of Hg will be approximately ten times higher than in the past exercise; v) the other two elements will be either Cr+Zn or Cu+Fe.

In order to avoid problems with the long-term storage of the solutions, analyses should be performed within two weeks from the receipt of samples in two separate analytical runs. Each run should consist of three individual measurements using the same calibration curve. Participants will receive the full assessment of data supplied at the end of each proficiency test.

It was also decided that the analysis of synthetic solutions cannot be the only way to improve the performance of the NRLs as these have to deal with a variety of matrices. Consensus was thus reached in the organization of an other proficiency test based on the determination of trace elements in fish tissue. The preparation of this test material, to be possibly used also as a candidate reference material will be the responsibility of the EC-JRC-IRMM on behalf of the ISS-CRL. In this case, the exercise will be conducted over one year and the laboratories will hence to quantify ten elements, namely As, Cd, Cr, Cu, Fe, Hg, Ni, Pb, Se and Zn.

Agenda item 7: Updating of the Handbook of analytical methods for trace elements in use at NRLs

An overview on the work done by the ISS-CRL over the last year to update, with the cooperation of the NRLs, the collection of the analytical methods in use for the determination of trace elements was presented by Dr. Alessandrelli (ISS-CRL). It was stressed that, among others, the role of the Handbook is to encourage the exchange of information among NRLs as regards the possibility of improving existing methods.

Agenda item 8: Any other business

The Chairman asked the audience whether there were additional comments or remarks about the items discussed during the meeting. No additional issues were raised.

Agenda item 9: Closure of the meeting

At 4.00 pm the Chairman thanked all participants and closed the meeting.